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THE EPIBRANCHIAL PLACODES OF LEPIDOSTEUS OSSEUS AND THEIR RELATION TO THE CEREBRAL GANGLIA

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FIFTY-EIGHT FIGURES

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INTRODUCTION

A description of the origin and fate of the epibranchial placodes of Lepidosteus was undertaken with the object of extending our knowledge of the part these placodes play in the formation of the cerebral ganglia. Ectodermic thickenings, in the

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region of the gill slits, concerned in the formation of the cerebral ganglia, have been known for a long time through the work of Beard ('85), Van Wijhe ('82), Froriep ('85) and others and seem to be present in all classes of vertebrates including the higher mammals.

The mode of origin of the placodes in many forms is such that it is difficult to determine whether there is an actual contribution of cells by the placode to the corresponding ganglion. In most types described, the neural crest which enters into the composition of a given ganglion such as the VII, IX and X, comes into contact at its ventral border with the epidermis just dorsal and posterior to the corresponding gill pocket, and it is difficult to determine just how far the contact is due to the lateral extension of the neural crest portion of the ganglion as contrasted with the mesial ingrowth of the cell mass derived from the thickening of the ectoderm or placode.

In some types such as the catfishes (Landacre, '10), however, the mode of origin of the epibranchial placodes is such that the conditions are easy to interpret. The epibranchial placodes of the VII, IX and first two branchial ganglia of the X arise free from contact with the endodermic gill pockets and become detached from the epidermis *en masse* and are added to the remaining portions of the cerebral ganglia in such a manner that they can be followed with ease up to the time they become fused with the neural crest ganglia. The last two branchial ganglia of the X do not appear until the neural crest ganglia in their downward growth approach the skin, and consequently do not furnish such good evidence of the integrity and continuity of their placodes. All these cell masses derived from the placodes, except in the case of the IX, become indistinguishably fused with the general visceral ganglia derived from the neural crest or its homologue in the lateral mass.

The condition of the epibranchial placode in the IX ganglion of Ameiurus is of the greatest importance in determining the significance of these structures. The visceral portion of this ganglion seems to come exclusively from the epibranchial placode

of the first true gill and the visceral portion of the ganglion is detached from the lateralis portion. Herrick ('07) found only special visceral or gustatory fibers arising from the visceral ganglion so that, since the visceral portion of the ganglion is exclusively placodal in origin, we are warranted in concluding that the epibranchial placodes give rise to those ganglionic cells from which gustatory fibers arise. This conclusion is strengthened by a number of facts that need not be repeated here. It does not seem an unwarranted conclusion that this is the function of the epibranchial placodes in all classes of vertebrates, although its demonstration in the higher vertebrates will always be a differcult undertaking, owing to the fact that the relations of the placode to the general visceral ganglion rarely seem to be so diagrammatic as in Ameiurus and that it is extemely difficult to secure a series of embryos of the higher vertebrates in stages sufficiently close to follow all the changes, since these series must in the lower forms, at least, be as close as four hours.

These facts emphasize the necessity of clearing the problem up as far as possible among the lower vertebrates, where series can be secured at sufficiently close intervals and where we should expect to find simpler relations owing to the generalized conditions of the peripheral nervous system. Of equal importance with the two facts just mentioned, however, is that of the hypertrophy of some of the components of the peripheral nerves, especially among the Ichthyopsida. This is probably the reason for the diagrammatic simplicity of the special visceral system of Ameiurus.

While Lepidosteus was taken up primarily because it is a generalized type, it appears that there is a beautiful example of hypertrophy in the case of the visceral portion of the VII ganglion as compared with the same ganglia in the IX and X. This is probably associated with the elongation of the head and the consequent increase in the area supplied by the VII nerve.

One of the principal difficulties encountered in the study of the placodes in Lepidosteus arises from the fact that the endodermal evagination from the pharynx is long anterio-posteriorly,

and seems to encroach upon the territory occupied by the ectodermic evagination forming the placode; at least the placode is so closely applied to the posterior surface of the pharyngeal pocket that it is often difficult and sometimes impossible to tell where one ends and the other begins. Aside from this feature the conditions are quite similar to those found in Ameiurus with the exception that the visceral portion of the IX is not purely placodal in origin and that the placode of the VII nerve is much larger in Lepidosteus than in Ameiurus. In view of these facts I shall describe the placode of the VII nerve in detail, and treat the remaining placodes briefly.

MATERIAL

The material consists of thirty-three stages taken at intervals of six hours from one lot of eggs. Usually this would be too long an interval to follow accurately the changes in the cerebral ganglia but, by cutting a large number of series of any given age, it is rare that one cannot pick out some one of a given series that is as far advanced or even further advanced than the youngest stage of the next older series; so that if the series are sufficiently numerous they become practically continuous. The sections were cut 6 μ thick and stained in bulk in Delafield's haematoxylin one-sixth the strength of the stock solution, for twenty-four hours. This gave a much better differentiated stain than when sections were stained on the slide and owing to the amount of yolk granules present is much superior to Heidenhain's stain.

Table 1 shows the age, length and increments in age and length of embryos of Lepidosteus osseus ranging from 100 hours after fertilization to 272 hours after fertilization. Several series younger than 100 hours are referred to in the body of the paper but are not included in the table because they had not been freed from the membranes before fixation and consequently could not be measured accurately. They are referred to by age only.

TABLE 1

Showing the age, length and increments in age and length of embryos of Lepidosteus osseus ranging from 100 hours after fertilization to 272 hours after fertilization.

	NUMBER OF SERIES																
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Age from fertilization in hrs.	100	106	112	120½	130	137	144	148	154	160	166	172	184	191	196	214	262
Increment in hrs.	6	6	6	8½	9½	7	7	4	6	6	6	6	12	7	5	18	48
Length in mm...	7	7.3	8	8.3	8.8	9.5	9.7	9.9	10	10.9	11.3	11.5	12.4	12.9	13	13.5	24
Increment in mm		0.3	0.7	0.3	0.5	0.7	0.2	0.2	0.1	0.9	0.4	0.2	0.9	0.5	0.1	0.5	10.5

THE 10 MM. EMBRYO (GENERAL)

Owing to the fact that the nerve components of the adult Lepidosteus have not been worked and that there are some peculiarities in the size and arrangement of the ganglia and nerves, it will simplify the description of the placodes to give a general account of structures and to give a rather complete account of the ganglia and nerves of some stage, for which I have selected the 10 mm. embryo.

This stage represents an intermediate condition in the formation of the epibranchial ganglia; the placodes being completely detached from the epidermis in the geniculate or VII ganglion, in process of formation in the IX or petrosal and first two epibranchial ganglia of the X, while the two remaining epibranchial placodes of the X are not yet present, there being only three well defined gill bars.

The general form of the anterior end of the body is quite characteristic as shown in fig. 1. The anterior end of the head has a sharp downward flexure beginning in the region of the infundibulum and ends in a prominent sucking disc. Directly over the posterior end of the infundibulum lies the mesencephalon which marks the highest point on the dorsal surface of the body.

The mouth is open and the pharynx has a cavity to a point just posterior to the third gill. Neither teeth not taste buds can be detected in the oral cavity or pharynx.

No cartilages are present at this stage, although the primordia of both cartilaginous and muscular structures can be identified in condensed masses of mesoderm.

The olfactory capsule has no cavity and is attached to the ectoderm throughout almost its whole length. Both roots of the olfactory nerve are well formed, that is, fibrillated and there are a number of loose cells lying around the nerve that probably develop later into the ganglion of the nervus terminalis. The optic vesicle is large, and elongated slightly in its anterior-posterior axis.

The auditory vesicle is a simple sac slightly elongated from anterior to posterior and has a short ductus endolymphaticus.

The supra-orbital, sub-orbital, mandibular and body sensory lines are present (figs. 6, 7, 15) and well defined and the position of some of the lateral line organs can be determined, although they are not well differentiated and not all of them are present, even on the head. The epidermal thickenings (figs. 8 and 9) forming the caudal extension of the epibranchial placodes are present and extend from the point of origin of the epibranchial placodes of the VII and IX nerves. They can be distinguished histologically from the primordia of the lateral lines in both the VII and IX nerves, and in the case of the IX, lie at a lower level than the primordia of the lateral lines.

The brain (fig. 1) at this stage shows a few of the peculiarities that are so pronounced in the adult Lepidosteus. The three primitive brain vesicles are still present. The telencephalon is not sharply separated dorsally from the diencephalon; in fact, except for arbitrary landmarks it is not possible to define the two divisions of the primitive prosencephalon. The epiphysis and dorsal sac are present at the boundary between the thin walled prosencephalon and thick walled mesencephalon, but the paraphysis is not present and appears first in an embryo of 11 5 mm. as a definite evagination, although its position can be determined somewhat earlier as a thickened area in the roof of the prosencephalon. With the appearance of the paraphysis comes the elevation and broadening of the thin roof of the telencephalon which marks it off as distinct from the diencephalon.

Ventrally the hypophysis is well developed and there is a marked flexure in the floor of the brain directly under the mesencephalon and directly over a point just anterior to the posterior end of the hypophysis. The pituitary body is well developed at this stage. The mesencephalon is well developed and rises considerably above the level of the roof of the brain and consists of a large flattened vesicle which extends laterally over the anterior end of the metencephalon and has uniformly thick walls (figs. 6 and 7). These lateral lobes of the mesencephalon completely conceal the anterior end of the metencephalon from the dorsal surface. The roof of the brain is of uniform thickness from the region of the epiphysis to the posterior end of the mesencephalon.

The anterior end of the metencephalon (figs. 6 and 7) consists of two lateral lobes with thick dorsal and ventral walls but with thin lateral walls. These, as mentioned above, are overhung by the broad flat mesencephalon. The anterior ends of these lobes are almost in contact with the posterior ends of the optic vesicles (fig. 1). At a point dorsal to the posterior end of the hypophysis they fuse on the median line and at a level with the entrance of the root of the Gasserian ganglion their cavities become confluent with the median ventricle. The roof of these vesicles is thick from their anterior end to a point just posterior to the posterior end of the mesencephalon. These two thick walled vesicles I take to be the lateral lobes of the cerebellum and the thickened roof of the anterior end of the metencephalon, lying under the posterior end of the mesencephalon and above the unpaired ventricle of the brain, the middle lobe of the cerebellum (valvula cerebelli). From the posterior end of the mesencephalon the roof of the metencephalon has the usual character of the medulla.

THE GANGLIA AND NERVES OF THE 10 MM. EMBRYO

Since the components of the cranial nerves in the adult Lepidosteus have not been worked out, certain difficulties present themselves when one attempts to identify the ganglia and nerves of the 10 mm. embryo. Some of these difficulties will appear in the description. For the most part, however, the identification of ganglia and nerves and even the identification of the components can be made with ease, owing to the isolated position of the ganglia and the solitary course pursued by components which are later combined into mixed nerve trunks. In the main, the position of definite ganglia is remarkably constant among the Ichthyopsida. The most striking differences among ganglia are three: (1) the presence or absence of the profundus ganglion; (2) the position of the ventro-lateral lateralis of the VII; and (3) the extent to which the branchial ganglia of the vagus are distinct. The profundus is present in Lepidosteus.

The ventro-lateral lat. is mesial to the geniculate, while it is lateral in the Amphibia (Coghill, '02); and lastly the branchial ganglia of the X, in contrast with the Amphibia, are fairly distinct.

The branchial ganglia of the 10 mm. embryo are in an intermediate stage, as stated above, the branchial ganglion of the VII being completely formed, or rather detached from the epidermis, and the last two branchial ganglia of the X not yet formed owing to the absence of the last two gills. The same statement can be made of the ganglia as a whole. The V, VII, VIII and IX ganglia are definitely outlined, while the posterior portion of the X, the visceral portion, is not definitely formed.

The general visceral and general somatic ganglia which arise from the neural crest pass through a stage when their cells are quite loosely arranged and their boundaries are indefinite, so that, during the early stages in the formation of neural crest ganglia it is difficult to determine the exact limits of their boundaries. The general visceral X is in this condition in the 10 mm. embryo. If one chooses an older embryo, however, in which the X ganglia are fully formed, the V and VII ganglia are fused to such an extent that it renders their description difficult unless the nerve components are readily separable by differences in size. This condition is not reached in a 6-inch Lepidosteus. The process of fusing of ganglia is particularly true in the case of the lateralis VII and the auditory. There is usually a brief period during the growth of these ganglia when their boundaries can be made out; but preceding and following this period the general visceral VII, lateralis VII, and auditory are likely to be more or less fused.

The profundus ganglion (ganglion mesencephali; trigeminus I)

This ganglion lies dorsal to the posterior portion of the optic vesicle (figs. 1, 4, 5). It is an elongated cord-like mass of cells placed diagonally in the mesoderm between the posterior portion of the optic vesicle and the mesencephalon. The anterior end

extends dorsal to the upper border of the vesicle and the posterior end lies at a lower level. The nerve trunk (ophthalmicus profundus) which arises at the anterior end of the ganglion can be followed forward at this stage to a point over the anterior end of the lens where it becomes so attenuated that it cannot be followed further.

The nerve forms a gentle curve corresponding to the dorsal surface of the optic vesicle and maintains a constant position over the middle of the optic vesicle. The ophthalmicus profundus does not at this stage come into contact with either the ophthalmicus superficialis VII or the ophthalmicus superficialis V and is presumably a pure general somatic nerve, although in the early stages of the formation of the ganglion (82 hours) there is a pronounced contact with the epidermis, but whether this is placodal in nature or not has not been determined. The root of the profundus arises from the posterior end of the ganglion and passes back and down arching under the lateral lobes of the metencephalon which it enters from the ventral surface. The root forms a gentle curve down and back reversing the relations of the trunk. At the middle of its course the root comes into intimate relations with the third nerve, and somewhat further posterior, with the ciliary ganglion also.

The Gasserian ganglion (trigeminus II)

This ganglion (fig. 1) in the 10 mm. embryo is a large oblong ganglion placed diagonally in the mesoderm with its anterior end at the side of the oral cavity and above the level of the roof of the oral cavity. It is nowhere in contact with the epidermis. It extends from a point just anterior to the pituitary body, where it is located under the posterior portion of the optic vesicle, back to a point at the level of the anterior end of the dorso-lateral portion of the lateralis VII ganglion. It is not in contact with either the profundus (trigeminus I), or the geniculate ganglion of the VII, nor with the dorso-lateral VII. The ganglion is compact and definitely outlined. Its root is at the posterior end and is quite short, the ganglion cells at this point being closely attached

to the ventro-lateral wall of the medulla at a point opposite the posterior end of the mesencephalic roof.

There are two well defined fibrillated nerves arising from this ganglion at this time, a supra-orbital trunk (ophthalmicus superficialis trigeminus), and an infra-orbital trunk (truncus infraorbitalis), which splits up into the maxillary and mandibular trunks.

The supra-orbital trunk (fig. 1) arises from the dorsal surface of the posterior end of the ganglion and runs upward and slightly forward and becomes so attenuated that it cannot be followed as far forward as the posterior border of the optic vesicle. There is consequently no connection between supra-orbital V and the profundus, such as we see later when these two nerve trunks come to lie closer together.

The infra-orbital trunk is much larger and longer. It arises from the extreme anterior and ventral end of the ganglion. It is separated from the point of origin of the supra-orbital trunk by the whole length of the ganglion. This condition is of course temporary, since the Gasserian ganglion becomes shorter as it becomes older, and the two points of origin are brought closer together. Near its point of origin the infra-orbital trunk divides into two branches, a dorsal, the ramus maxillaris V (figs. 4 and 5) and a ventral, the ramus mandibularis V. The ramus maxillaris pursues a course forward under the optic vesicle at the level of its point of origin and can be followed to the anterior border of the vesicle. The ramus mandibularis V runs directly ventral into the mandible. All the nerves arising from the Gasserian ganglion, owing to their freedom from contact with other nerves and ganglia, are at this time pure general somatic nerves.

The VII ganglion is composed, as usual among Ichthyopsida, of three more or less distinct masses; a visceral ganglion, the geniculate, and two lateralis ganglia, the dorso-lateral and ventrolateral. There is more or less fusion between these ganglionic masses as well as with the auditory ganglion at various stages of their growth and migration into the adult condition; so that it will be easier to describe them in the order of their simplicity.

The dorso-lateral VII

This ganglion (figs. 1, 8, 9) is an elongated rod-like mass occupying a position at the side of the anterior end of the medulla on a level with the floor of the medulla and between it and the epidermis. Its anterior end reaches as far forward as the root of the Gasserian, being situated of course more laterally, and its posterior end reaches posterior to the anterior end of the auditory vesicle and lies between the auditory vesicle and the medulla. The anterior end of the ganglion lies near the skin, while the posterior end lies near the cord, so that on a frontal section, it has a position diagonal to the longitudinal axis of the body. Its root enters the medulla along with the roots of the geniculate and ventro-lateral ganglion. It maintains about the same level dorso-ventrally throughout its course lying parallel with the long axis of the body. The posterior end of the ganglion comes into close contact with the posterior end of the geniculate and the anterior end of the auditory, the root fibers passing dorsally from the cells along the anterior end of the auditory ganglion.

There are three fibrillated nerves arising from this ganglion at this stage; a supra-orbital ramus, an infra-orbital ramus, and the ramus oticus. The first two arise from the anterior end of the ganglion and shortly beyond their origin from the ganglion come closely into contact with the skin, where they innervate lateral line organs anterior to the position of the ganglion. Neither of these rami comes into close relation with the corresponding rami of the Gasserian, so that we do not have true supra-orbital and infra-orbital trunks at this time. The supra-orbital ramus (lateralis portion of ramus ophthalmicus superficialis VII, figs. 4 to 7) arises from the dorsal portion of the anterior end of the ganglion, pursues a course diagonally forward and upward innervating lateral line organs of the supra-orbital line. It arches over the optic vesicle and can be traced with certainty as a fibrillated root to the anterior end of the optic vesicle. It always occupies a position quite close to the epidermis.

The infra-orbital ramus, ramus buccalis (figs. 4 to 7), originates from the ventral portion of the anterior end of the ganglion and pursues a course downward and forward in close contact with the skin where it can be followed as a fibrillated cord to a point near the anterior end of the optic vesicle. It innervates lateral line organs of the infra-orbital line anterior to the position of the ganglion. The ramus oticus is represented by a small twig arising midway between the anterior and posterior ends of the ganglion. It supplies the last lateral line organ in the infra-orbital line. The twig runs laterally from the ganglion and passes under the anterior end of the auditory capsule and can be followed easily throughout its whole course at this stage.

The geniculate ganglion

The geniculate ganglion is an elongated mass of cells placed diagonally in the body with the anterior end situated somewhat more ventrally and lying directly on the dorsal surface of the pharyngeal pocket (figs. 1 and 8). The anterior end of the ganglion lies as far forward as the posterior end of the Gasserian. The posterior end of the ganglion rises to the level of the base of the medulla and its root enters the medulla along with those of the dorso-lateral and ventro-lateral ganglia.

It is throughout part of its extent (fig. 9) wedged in between the dorso-lateral ganglion and the ventro-lateral ganglion, to be described in the next section, and its posterior end comes closely into contact with the auditory. The geniculate ganglion is double in composition. The posterior portion, derived from the neural crest, is definitely outlined and circular in form and seems to be purely general visceral in composition. The anterior end, which contains cells derived from the placode, is less regular in form and incloses or has attached to its ventral surface and resting directly upon the endoderm of the pharyngeal pocket a mass of cells which projects laterally giving a "comma" shape to the ganglion in transverse section(fig. 8). The laterally projecting mass is derived from the epibranchial placode and can be distinguished both by its color and position of its cells. The

placodal cells represent the special visceral portion of the ganglion while the remainder is general visceral. This placode is to be followed in detail later as well as similar cells in the IX and X, and will not be described more fully here.

Two nerves arise from this ganglion. The truncus hyomandibularis arises from the ventral border of the ganglion about one-third of its length from the anterior end and runs ventrolaterally from its point of origin.

The truncus hyomandibularis contains lateralis fibers derived from the ventro-lateral ganglion in addition to those derived from the geniculate. At the level of the floor of the pharynx it divides into two rami, the dorsal (ramus mandibularis) turning cephalad and the ventral ramus (ramus hyoideus facialis) running directly ventral.

A second smaller nerve (the ramus palatinus facialis) arises from the anterior end of the ganglion and pursues the course usual in teleosts following the roof of the pharynx and oral cavity forward to the level of the olfactory capsule. It seems to be accompanied throughout the proximal part of its course by the motor trunk of the facialis which supplies the muscle adductor arcus palatini and the relation of the two components at their exit from the ganglion is somewhat peculiar, in some series the visceral fibers having the appearance of entering the lateral line ganglion (ventro-lateral). In other series of the same age, however, one can trace the visceral component into the anterior end of the geniculate while the motor component passes further caudad and enters the ganglion from the ventro-mesial side where it joins the motor trunk running out with the truncus hyomandibularis.

The ventro-lateral VII

This ganglion (figs. 1 and 9), as I have identified it, lies on the mesial side of the anterior third of the geniculate, partially inclosed in a crescent shaped depression of the geniculate. This ganglion has a very characteristic appearance. The interior of the ganglionic mass is usually free from nuclei, all the cells being arranged radially with the small ends directed centrally (fig. 29).

The whole ganglion in embryos of 10 mm. is not usually more than one-third as long as the geniculate, but this ratio varies, since the ganglion is sometimes triangular with the smaller end extending back into the truncus hyomandibularis. In older embryos the ganglion is always triangular and the posterior end grows posterior and ventral to the geniculate, thus simulating the relations in Menidia (Herrick, '99), although the greater portion of the ganglion retains its position mesial to the geniculate. The fibers coming from the ganglion, mentioned in the preceding section as running out in the hyomandibular nerve, arise from the extreme posterior end of the ganglion and run into the hyomandibular on the ventral surface of the visceral fibers. The root arises here also and arches around the mesial surface of the geniculate ganglion and enters the medulla along with those of the dorso-lateral ganglion.

The position of the ventro-lateral ganglion in Lepidosteus differs from the position in Menidia (Herrick, '99) and in the embryo of Ameiurus (Landacre, '10) and in the Amphibia Coghill ('02 and '06). In the embryo of Ameiurus (56 hours) the ventro-lateral ganglion lies posterior to the geniculate, in Menidia posterior and mesial, while in the urodeles it is lateral (external) to the geniculate. There seems to be no other case recorded where it is directly mesial to the geniculate.

The fact that the nerve components have not been worked and consequently one cannot trace the ganglion back to this stage from an older series, renders the identification of this ganglion more difficult than in such cases as the Gasserian and dorso-lateral, where the ganglia are distinct and their nerves contain only one component which can be traced to its peripheral distribution.

Since, however, the placodal portion of the geniculate is quite distinct and its history can be traced, it becomes an important factor in the differentiation of the geniculate and ventro-lateral ganglia. Notwithstanding the rather unusual position of the ganglion identified as ventro-lateral, the following facts seem to warrant the identification:

(a). The two ganglia are quite distinct histologically.

(b). The epibranchial placode is added to the one identified as geniculate, or general visceral, as in the IX and four branchial ganglia of the X.

(c). The ramus palatinus, which does not contain lateralis fibers in any type, arises from the geniculate.

(d). The ganglion identified as ventro-lateral seems to send all its fibers into the hyomandibular.

(e). In the later stages of the ventro-lateral ganglion it assumes a position more ventral and posterior to the geniculate as in Menidia and in Ameiurus.

The auditory ganglion

The auditory ganglion (fig. 1) is a large comma-shaped mass with the large end directed forward and the smaller end extending caudad. The large anterior end is closely attached to the posterior end of the geniculate and to the ventro-lateral VII. The root enters the medulla from the anterior end and a fibrillated trunk enters the auditory capsule at the middle region of the ganglion.

The glossopharyngeus ganglion (petrosal and lateralis portion)

This ganglion (figs. 1 and 10) is club-shaped with the large end projecting forward and downward and is attached at its extreme anterior end to a mass of cells (placode II) proliferated from the ectoderm at the posterior and dorsal portion of the pharyngeal pocket of the first true gill. The posterior attenuated portion arches up around the posterior surface of the auditory capsule and enters the medulla at a point almost directly dorsal to the anterior end of the ganglion. Just before it enters the medulla it passes into a well defined mass of cells. This is not a ganglion however, since in later stages there are no ganglion cells in this position and the thickness of the root at this point as shown in fig. 1 is undoubtedly due to the exit of motor fibers and the presence of the root of the lateralis X ganglion which enters at this point. After the lateralis X root becomes more completely

medullated the appearance of the proximal portion of the root of the IX changes completely and there is no connection between IX and X except through the lateralis X root which runs forward as in Ameiurus from the lateralis X ganglion to enter near the entrance of the IX root.

The glossopharyngeal ganglion contains three components, (1) the general visceral, (2) the placodal (special visceral or gustatory) and (3) lateralis cells. The lateralis portion of IX, in the 10 mm. stage and in preceding stages, is quite distinct in outline from the visceral portion and occupies the dorso-lateral portion of the IX and can be positively identified by the ramus supratemporalis which innervates a lateral line organ lying just lateral to the ganglion in the same transverse level. The anterior portion of the ganglion is composed of general visceral cells derived from the neural crest, and of the placodal portion which can be distinguished both by color and form of cells, while the posterior portion is composed of the general visceral cells and lateralis cells. Two nerves are present at this stage; the truncus glossopharyngeus, arising from the anterior end of the ganglion and running ventrally into the first gill bar and containing probably both general and special visceral fibers, and a lateral line nerve, ramus supra-temporalis, arising from the middle of the ganglion and running laterally to innervate at least one lateral line organ of the body line which I take to be the second organ of this line.

The vagus ganglia

This complex (fig. 1) contains at this time three chief ganglionic masses (a) the lateralis X ganglion, (b) the first branchial ganglion and (c) a large poorly differentiated ganglion corresponding to the primordia of the general visceral (nodosal) and three remaining branchial ganglia. This last mass comes into contact with the third gill slit and contains cells contributed by the third placode (counting the placode of the VII as no. 1) and its posterior end is not clearly definable. There is no definite jugular, or general somatic, ganglion present at this time. It can be detected first in my series in a 13 mm. embryo as a

small ganglion lying on the root of the vagus intracranially. At this time its ganglionic cells cannot be distinguished from embryonic sheath cells.

The lateralis X

The lateralis X is the best defined ganglion of the vagus group. It is an elongated cylindrical mass of cells with its anterior end reaching the posterior end of the first branchial ganglion of the vagus and its posterior end reaching posterior to the caudal end of the visceral X. It is situated laterally in the body at the level of the notochord and lies between the skin and the branchial ganglia of the vagus. I can detect only two fibrillated nerves arising from it at this time. The chief nerve (ramus lateralis vagi), arises from the posterior end of the ganglion and runs posteriorly as usual in teleosts. The second nerve is the ramus supra-temporalis vagi which arises from the anterior end of the lateralis X ganglion and passes directly lateral and slightly forward to a lateral line organ apparently the third organ of the body lateral line.

The root of this ganglion does not enter with the vagus roots but pursues a course diagonally forward and upward to the region of the root of the glossopharyngus with which at this stage, it enters the medulla. In older embryos the fibrillated root of the lateralis X passes beyond the visceral root of the IX and enters the medulla anterior to that root.

The branchial ganglia of the vagus

The first branchial ganglion of the vagus (figs. 1 and 11) is a visceral ganglion composed of cells derived from the neural crest (general visceral) and of cells derived from the placode of the second true gill (special visceral or gustatory). In shape it resembles the glossopharyngeal but has a position more nearly vertical in the body. The ventral end is the larger and it curves dorsally and at this stage comes into contact at its dorsal and posterior border with the remaining portion of the general visceral mass of the X. It is convex on its anterior face. The

root of this ganglion enters the medulla along with the remaining visceral roots of the X. One fibrillated nerve is present (truncus branchialis vagi I). It arises from the anterior ventral end of the ganglion just behind its attachment to the placode and runs ventrally into the second true gill bar.

The remainder of the visceral X is not differentiated into branchial ganglia. It is an elongated mass of cells lying somewhat ventrally and mesially to the lateralis X. Its posterior end as mentioned above is ill defined. Its middle portion is attached to the placode (placode 4) and one branchial nerve (truncus branchialis vagi II) arises from it just posterior to its attachment to the placode. This ganglionic mass differentiates later into the three remaining branchial ganglia of the X having the same composition as the first branchial of the X and consists of general visceral cells derived from the neural crest and special visceral or gustatory cells derived from the placodes.

The root of this ganglion passes dorsally along with that of the first branchial ganglion and part of its fibers enter the medulla directly over the ganglion. Many of the fibers, however, at this stage pass further posterior forming a bundle lying near the medulla and enter the medulla posterior to the posterior end of the ganglion.

DETAILED DESCRIPTION OF THE EPIBRANCHIAL PLACODE OF THE VII IN THE 10 MM. EMBRYO

This placode is completely detached from the epidermis in the 10 mm. embryo, as mentioned above. After describing it in this stage its later history will be followed first, and then the mode of origin will be taken up.

After detachment from the epidermis, the placode occupies the anterior end of the general visceral ganglion of the VII nerve (fig. 1). The posterior and middle portions of the geniculate are round in transverse sections. The anterior third is indented on its mesial side by the ventro-lateral lateralis ganglion (figs. 20, 21 and 27). The extreme anterior end varies a good deal in shape in different series owing to the varying relations of the

general visceral and placodal portions to each other. The general visceral portion sometimes extends farther forward than the placodal portion, sometimes both general visceral and placodal portions are of equal length and the anterior end of the ganglion is split (figs. 14, 15). In all series of this stage the anterior end consists of a mesial rounded portion and of a lateral spur resting on the endoderm of the hyoid gill pocket and extending somewhat dorso-laterally toward the epidermis (figs. 16 to 20). A portion of the median rounded mass and all of the lateral spur are derived from the placode. The manner in which the placodal portion joins the general visceral varies in different embryos somewhat. The visceral cells may lie like a cap dorsal, mesial and sometimes lateral to the placodal cells. In later stages the placodal cells are usually surrounded by the general visceral cells in this manner, but the condition shown in figs. 14 to 21 is the usual one in the 10 mm. stage, so that the placodal portion of the ganglion begins at the extreme anterior end of the ganglionic mass (fig. 14) becomes broader a few sections posterior to this point and possesses a large lateral spur (figs. 17 to 19) and disappears a few sections posterior to the anterior end of the ventro-lateral lateralis VII.

The placodal portion of the ganglion can be distinguished from the general visceral by the difference in staining reaction, the placodal portion usually being much darker (fig. 26) and can be distinguished further, by the arrangement of the cells, the placodal cells being elongated in the direction in which they have moved into the ganglionic mass. The most uniform and most easily recognized characteristic is that of color (figs. 26, 28, 30, 31).

Posterior to the placodal portion of the geniculate ganglion we find the general visceral and ventro-lateral and dorso-lateral portions only (fig. 27). The ventro-lateral ganglion lies mesial and quite close to the geniculate and usually imbedded in it. It is a short round ganglionic mass with its cells usually arranged in a rosette with the nuclei situated peripherally. The geniculate ganglion has a totally different appearance, consisting of a rather dense mass of cells irregularly arranged and having very indefin-

ite cell boundaries (figs. 26, 27, 28). Posterior to the ventro-lateral lateralis ganglion, the geniculate is uniform in structure and passes dorsally and posteriorly toward the medulla accompanied by the root of the ventro-lateral ganglion. The posterior end of the geniculate ganglion is closely attached to the auditory ganglion, and near its entrance into the medulla, is joined by the root of the dorso-lateral lateralis ganglion; so that all the roots of the VII ganglion come into contact with the medulla near the same point and quite close to the entrance of the root of the auditory. The ganglionic complex of the VII has in general an arrangement quite characteristic for the Ichthyopsida (Herrick, '99, Landacre, '10) with the exception that it is quite easy at this stage in the development of Lepidosteus to separate the placodal or special visceral component from the neural crest or general visceral portion of the geniculate.

The relation of the placodal portion of the VII ganglion to the endoderm of the hyoid gill pocket is of the greatest importance in forming a clear conception of the mode of origin and detachment of the placode. The hyoid gill pocket as it approaches the ectoderm gives off at this stage two solid processes of cells neither of which come into contact with the epidermis (figs. 14 to 16), but from an early stage up to the 8.8 mm. stage both these processes are in contact with the epidermis. As they withdraw from the epidermis there appears a rather dense mass of mesoderm between the two processes and in the area between the processes and the ectoderm (fig. 19). This mass of mesoderm later develops into the hyomandibular cartilage and muscles associated with this cartilage and the ear capsule. The dorsal process lies farther forward, considerably anterior to the anterior end of the geniculate ganglion. At the anterior end of the geniculate it has the appearance shown in figs. 14, 15, 16. The primordium of the hyomandibular cartilage lies between the hyoid pocket and the epidermis in a recess formed by the dorsal and ventral prolongations of the endoderm and extends forward from this point. Four sections posterior to fig. 14 the ventral prolongation becomes detached and seems to disappear later, at the same time the dorsal prolongation becomes shorter and seems to detach a mass of

cells which also disappear later. The placodal portion of the ganglion always rests upon the dorsal endodermic prolongation and in earlier stages is in continuity with the ectoderm at the posterior end of the dorsal endoderm process and abuts against it as shown in model (fig. 33). In the 10 mm. embryo where the placode is no longer in contact with the ectoderm and is separated from it by the primordium of the hyomandibular cartilage it still rests upon this dorsal pocket. The later history of the placodal cells is closely associated with this pocket.

THE LATER HISTORY OF THE PLACODE OF THE VII

The later history of the placode may be summarized briefly as consisting of (a) the withdrawal from the epidermis and incorporation into the geniculate ganglion, and (b) the reduction in number and the metamorphosis of the numerous compact placodal cells into ordinary ganglion cells that cannot be distinguished from cells of the geniculate ganglion derived from the neural crest. As to the withdrawal from the epidermis, a comparison of figs. 22, 23, 24, 25, with figs. 17, 18, 19 will show that the placodal spur of the geniculate ganglion nowhere approaches so closely to the epidermis as in the earlier stage. Fig. 25 shows also that the posterior end of the lateral spur extends caudad from the ganglion and lies, as an elongated mass of cells, small and dark staining like the placode, upon the endoderm of the hyoid pocket. The appearance of the posterior extension of cells is usually like that in fig. 25. The appearance of the caudal prolongation changes slowly. It is gradually withdrawn into the ganglionic mass of the geniculate. Fig. 30 illustrates the condition at the middle of the placodal mass and fig. 29 illustrates the condition five sections caudad where there are no placodal cells in the ganglion but the posterior spur is present (12.4 mm.). In stage 13.5 mm. there is no longer any posterior projection of placodal cells although the placodal cells reach the ventral surface of the ganglion (fig. 31).

The later history of the placodal cells is rather peculiar. They are the most striking feature of the facialis complex. The cells

as mentioned above stain a deep blue and in addition are quite small and closely packed. In a 24 mm. embryo they can be identified still, although reduced in numbers, and they seem to be represented in a 44 mm. and even in a 6-inch Lepidosteus. That they should remain so long undifferentiated is striking, but there can be no doubt as to the continuity of this mass of cells and that they are derived from the placode. That they are gradually transformed into ganglion cells seems evident from the conditions in the older series where they are of various sizes and some of them are evidently ganglion cells.

In a 24 mm. embryo the number of undifferentiated placodal cells is much reduced and there are groups of similar small cells in the posterior portion of the Gasserian, so that the minute cells are not peculiar to the placodal ganglion. The process of differentiation seems to take place on the periphery of the placodal cells where they are in contact with the normal ganglion cells. Since some placodal cells remain undifferentiated up to the 6-inch stage, it is possible that they may never be converted into normal cells, although this is improbable. The transformation of these placodal cells into ganglion cells is roughly correlated with the relative time of appearance of the placodal ganglia. They are the last ganglia to be formed and it is not surprising that they should transform into normal cells much later, but it is a little surprising that they should be delayed so long in their transformation.

THE ORIGIN OF THE EPIBRANCHIAL PLACODE OF THE VII

The determination of the exact time of appearance of the epibranchial placode of the VII nerve is rendered difficult by the presence of three associated structures, viz; the preauditory placode, the thickening of the epidermis at the point where the hyoid endodermic gill pocket joins the epidermis, and lastly the presence of the anterior end of the geniculate ganglion which ends at the point where the epibranchial placode is formed, rendering difficult the identification of detached groups of cells lying in this immediate region.

The preauditory placode is the first of these to appear and consists, as in Ameiurus (Landacre, '10), of a forward continuation of the thickening of the epidermis which forms the auditory vesicle. This thickening (including preauditory placode, auditory vesicle, and post-auditory placode) in its early stages is much longer than the auditory vesicle which is formed in its middle region and its anterior end can be traced forward in the epidermis as a thickened column of cells to the region of the hyoid gill pocket. As in Ameiurus, this preauditory placode is modified in its anterior end and seems to disappear as a preauditory placode, but the posterior portion of it persists to a relatively late stage and is closely associated with, although probably not genetically related to, the epibranchial placode of the VII nerve. Before the appearance of a placode, or at least before the proliferation of cells begins to form the epibranchial placode, the preauditory placode seems to be continuous at its anterior end with the thickening of the epidermis at the point of contact of the endoderm of the hyoid gill pocket with the ectoderm. The preauditory placode (which is presumably the earliest trace of the dorsolateral placodes of the authors) seems to be continuous with the ectodermic thickening of the gill pocket up to 82 hours, but no thickening of either gill pocket or preauditory placode extends beyond the hyoid gill pocket.

The preauditory placode shows, in its posterior part particularly, an arrangement of its cells characteristic of early stages in the auditory vesicle and of lateral line organs. The cells are radially arranged but the placode does not show these characteristics as it approaches the hyoid gill pocket (fig. 35).

The thickening of the epidermis at the point where the endoderm of the hyoid gill pocket joins the ectoderm is the most conspicuous feature of the hyoid region up to the time that the epibranchial placode appears (fig. 34). The significance of this thickening is uncertain. It may be due simply to the stimulus furnished by the contact of the endoderm with the epidermis, but is much more pronounced than in Ameiurus and consequently obscures the early differentiation of the epibranchial placode as well as renders it difficult to determine the exact relation of the

preauditory placode to both the epibranchial placode and the supra- and infra-orbital lines.

The cell arrangement of this thickening presents certain definite histological characters, the most conspicuous of which is the fact that it can be distinguished readily from the endoderm by the darker stain taken by the ectoderm, apparently due to the smaller size of the cells, the more compact and irregular arrangement, and the earlier loss of definite cell boundaries. These characters make it easy to trace the line of demarkation in most preparations between endoderm and ectoderm.

The third structure that must be constantly kept in mind in tracing the history of the placode is the position of the anterior end of the general visceral (geniculate) portion of the VII ganglion. This mass of cells, indefinite in outline as are all neural crest ganglia in their early stages, lies at the level of the hyoid gill pocket between the posterior end of this structure and the auditory vesicle and extends forward from the auditory vesicle to the posterior portion of the endodermic gill pocket. It is thus seen to be a mass of cells whose anterior end is wedged into the pocket formed by the withdrawal of the posterior end of the gill pocket from the ectoderm, and it is at this exact point that the ectoderm proliferates cells mesially to form the placode; so that the difficulty that has been found to exist in the interpretation of the relation of placodal cells to neural crest cells in other types occurs here with the exception that one can be certain that the neural crest portion of the VII (general visceral ganglion) does not form a contact with the epidermis. The difficulty arises in determining if a given group of cells which one finds in the anterior end of the geniculate came from the forward extension of the geniculate or from the placode. When these cells come off *en masse* and are numerous, no difficulty is presented but the determination of the source of small groups of cells, as in fig. 37, does present a difficulty. In the later stages of the formation, the placodal cells are detached from the epidermis in a large compact cluster, which can be distinguished from neural crest cells, so that the tracing of their ancestry is easy; but in the early stages of the formation of the geniculate ganglion this

is not true owing to the indefinite outlines of both the placode and general visceral ganglion.

Since the placode begins by the proliferation of cells mesially at the posterior end of the hyoid gill pocket and at the anterior end of the preauditory placode, it can be seen readily that it is only when the process of prolification has reached a somewhat advanced stage that the placode can be definitely identified. Conditions in Ameiurus are somewhat simpler (Landacre, '10). There the preauditory placode does not persist in the region of the hyoid pocket up to so late a period and there is a definite histological change in the region of the pocket before the process of proliferation begins, and further there is no such marked thickening of the epidermis in the region of contact of ectoderm and endoderm as in Lepidosteus. However, aside from the presence of pronounced sensory lines, the primordia of the lateral lines, there is no striking difference between the two types, the persistence of the anterior end of the preauditory placode and the great thickness of the ectoderm being minor differences.

A fourth structure, the posterior extension of the epibranchial placode, should be mentioned in connection with the three just described. In the case of the IX and Xth ganglia it presents no difficulties, since the posterior extension of the epibranchial placode lies at a lower level and is quite distinct from the dorsolateral placode or primordia of the lateral lines.

In the VII, however, this is not true. The posterior extension of the epibranchial placode lies in the same plane approximately as the preauditory placode. In the early stages of the epibranchial placode the preauditory placode extends forward to the hyoid gill. Later, as the preauditory placode recedes toward the ear, the epibranchial extends backward toward the ear in the same plane as that occupied by the preauditory.

The most probable interpretation of the relation of the preauditory placode to the ectodermic thickening of the gill pocket, and of the epibranchial placode to both of these, is that the preauditory placode extends forward into the thickening of the ectoderm in the hyoid region and before the disappearance of the preauditory placode and during the maximum development of the

ectodermic thickening the epibranchial placode appears. The later history of all three structures almost precludes the interpretation that their relations to each other involves more than juxtaposition.

The first probable trace of the appearance of the epibranchial placode is found in a 94-hour embryo. A section taken through the extreme posterior end of the contact of the hyoid endodermic gill pocket with the ectoderm (fig. 36) shows that the ectodermic thickening extends further mesially than it does anterior to this point and farther than in preceding stages. One section posterior to this (fig. 37) the endoderm has withdrawn completely from the ectoderm but the thickening is present and is continuous with an irregular mass of cells lying between the placode and endoderm. The proliferated mass of cells is continuous posteriorly with the neural crest (general visceral) portion of the VII. Mitotic figures are numerous between the cell mass and the placode indicating its origin from the placode. If this is true, however, the anterior end of the geniculate consists almost exclusively of cells derived from the placode since there is added to these cells later, the large body of cells that comes off *en masse* and is much more definite in outline.

In a 7.3 mm. embryo the epibranchial placode has reached a stage in its development such that it can be positively identified as the structure that later becomes detached *en masse* and added to the general visceral portion of the VII, since there is no break in its continuity from this stage up to the time of its detachment from the ectoderm. The ectoderm in the region of the placode is definitely differentiated into the primordia of the sensory lines, placode, and the thickening of the ectoderm at the attachment of the endodermic pocket. Throughout the length of the attachment of the endodermic pocket, and dorsal to the thickening of the ectoderm associated with this pocket, there is a second thickening incorporated more or less with the ventral thickening but distinguished from it by its rounded contour and by the arrangement of its cells. This dorsal thickening (fig. 38) is the primordium of the sensory lines anterior to the hyoid gill pocket. This line at this stage extends somewhat anterior to the hyoid

gill pocket and also extends posterior to this point (figs. 39, 40); so that it extends backward past the point of origin of the placode.

The dorsal sensory line shown in figs. 39 and 40 extends one-half of the distance from the hyoid gill pocket to the auditory vesicle, while the thickened area on which the placode forms extends two-thirds of this distance. This series is unusual, since in later series I cannot find a dorsal sensory line extending posterior to the placode and lying dorsal to it.

The form and position of the placode is shown in fig. 39. It consists of a well defined mass of cells projecting mesially and lying just ventral to the dorsal sensory line. Fig. 40 is taken four sections posterior to fig. 39 and shows the posterior extension of both the sensory line and the thickening which represents the posterior extension of the epibranchial placode and is continuous with the preauditory placode. The anterior end of the general visceral VII is more definite in outline than in the preceding stage figured, and comes into contact at its anterior end with the placode, although there is no difficulty in separating the two structures owing to the definite outline of the placode. There is the doubt, however, as to the composition of this extreme anterior end of the general visceral ganglion mentioned above.

The changes in an 8 mm. embryo are not marked, consisting chiefly in the increase in size of the mass of cells proliferated mesially in the placode. It should be borne in mind that the anterio-posterior extent of the placode is usually not over three or four sections (24μ) thick, so that in transverse sections one passes from the endodermic prolongations of the hyoid pocket directly into the ectodermic prolongation of the placode. This thickness in the anterio-posterior extent of the placode seems to be due to its being apposed so closely to the posterior surface of the gill pocket and gives the placode when seen cut through its greatest transverse dimension, the appearance of being much larger than it is.

In fig. 41 the endoderm of the gill pocket characterized by its pale color and large cells with definite walls abuts directly against the dark staining small celled ectoderm so that the boundary line is quite definite. In fig. 41 the continuity of the gill pocket

and ectoderm is not broken but at least half of this is formed of ectoderm and the ectodermic cells at the base of the proliferated mass are in active mitosis.

In fig. 42 the endodermic pocket has withdrawn from the ectoderm and the placodal mass projects freely into the mesoderm. One section posterior to this point the placodal mass is shorter and posterior to this is present as a slight ridge in the ectoderm. The condition just described, i.e., a thin flat placodal proliferation of cells closely apposed on its anterior surface to the posterior wall of the hyoid gill pocket and usually extending through not more than four sections, is so constant that it is unnecessary to follow it in detail further than to give sections through the placode at its maximum size. Between the 7.3 mm. stage and the 8 mm. stage the general visceral portion of the VII has grown forward as shown in fig. 42 until it lies upon the dorsal surface of the posterior end of the hyoid gill pocket. This change in position of the anterior end of the ganglion carries the ganglion anterior to the point at which the placode originates; so that the placodal cells are added in such a manner that they work their way into the general visceral mass or are surrounded by the visceral cells; at any rate they are incorporated into the general visceral ganglion.

In the 8.3 mm. stage (fig. 43), both the placode and the anterior end of the general visceral VII have increased in size. The general visceral ganglion is irregular in outline and it is difficult in this particular series to tell how much of the complex is placodal. Mitotic figures are numerous both in the ganglion and in the placode. The dorso-lateral VII ganglion is present in fig. 43 but the section is taken just anterior to the ventro-lateral VII.

The changes in the placode, aside from its darker color, are almost imperceptible between the 8.3 mm. stage and the 9.5 mm. stage (figs. 32 and 33). The general visceral portion of the geniculate, however, becomes more definite in outline and compared with the placodal portion of the ganglion, is much lighter in color and the cells are larger and more loosely arranged. Between the 9.5 mm. stage and the 10 mm. stage which has been fully described, the placode becomes completely detached from

the ectoderm in most series. In the process of becoming detached the placode seems to break near the epidermis and does not leave the epidermis of normal thickness at the point of detachment. The presence of loose masses of mesoderm cells in the region of the placode and particularly the presence of the primordium of the hyomandibular cartilage and associated muscles render it difficult to determine the exact time of detachment even though it were constant. The thickening of the epidermis after the placode is completely detached extends from the posterior end of the placode to the level of the anterior border of the auditory capsule. While this thickening does not confuse the relation in the hyoid region during the later stages of the placode, it is of the greatest importance to determine its early history and exact anatomical relations with reference to the dorso-lateral sensory lines.

SUMMARY OF THE HISTORY OF THE FIRST EPIBRANCHIAL PLACODE

The epibranchial placode of the VII nerve in Lepidosteus can be identified first as a definite structure whose history can be followed consecutively, at about the age of 94 hours after fertilization. It appears as a mesial proliferation of cells from the ectoderm just posterior to the endodermic pocket of the hyoid gill to whose posterior surface it is closely attached. The placode appears to be the posterior portion of the ectodermic thickening with which the endodermic hyoid pocket comes into contact. It is also apparently continuous posteriorly with a thickened ridge of cells, the preauditory placode, extending back to and in early stages being continuous with the anterior end of the auditory vesicle. Both the preauditory placode and hyoid ectodermic thickening are present before the placode appears and they are continuous with each other. As the placode grows mesially it comes into contact with the anterior end of the general visceral portion of the geniculate ganglion, with which it fuses, sometimes being attached on the lateral portion of the geniculate, sometimes to the ventral portion, and sometimes being

surrounded by general visceral cells. After growing mesially and coming into contact with the general visceral ganglion at about the age of 94 to 100 hours, it becomes detached from the ectoderm when the embryo is somewhat less than 10 mm. in length. At the point of detachment there is left a cell mass which extends backward to the region of the ear.

Preceding and for a long time after detachment, the placodal cells are sharply differentiated from the remainder of the geniculate cells. As late as the 44 mm. stage and probably as late as the 152 mm. stage some of the placodal cells are differentiated by their small size and intense dark color. The number is constantly reduced, however, and similar small dark staining cells can be detected in other ganglia particularly the Gasserian, so that the fact that one can trace the history of these cells to so late a period is probably due to their late differentiation into normal ganglion cells.

Just how much of the geniculate ganglion at any stage after the detachment of the placode and after the metamorphosis of the small placodal cells into normal ganglion cells, may be general visceral and how much may be special visceral cannot be determined, but the late differentiation of these placodal cells enables one to locate them and follow their history before metamorphosis much more completely than in any type described.

The relation of the early stage of this placode to the dorso-lateral sensory lines needs a more careful study than can be given the question in connection with the later history of the placodes. The presence of definite sensory lines resembling those of the sea bass (Wilson, '91) shows that the lateral line system, at least in its earlier stages, resembles Serranus much more closely than Ameiurus, but whether these sensory lines are continuous with the anterior end of the auditory thickening (preauditory placode of Ameiurus, Landacre, '10) or not must be taken up separately. The presence of epidermal thickenings or sensory lines, lying at the same level as the preauditory placode or dorso-lateral sensory line, but not identical with it, as a study of the epibranchial placodes of the IX and X nerves shows, emphasizes the necessity of exercising caution in order not to confuse these dorso-lateral

sensory lines with ventro-lateral sensory lines when they lie at the same level and apparently are continuous the one with the other.

THE EPIBRANCHIAL PLACODE OF THE IX NERVE

The placode of the IX nerve resembles the placode of the VII in its mode of origin, detachment, and incorporation into the general visceral ganglion. It is much smaller, however, and occupies less time in the transition from ectoderm into ganglion cells. The difficulty in determining the time of appearance of the placode of the IX is in part the same as that encountered in the VII. Owing to the angle at which the gill meets the roof of the pharynx, the ectodermic invagination in the region of the IX is more extensive and projects further caudad relatively than in the VII and until there is some structural or color differentiation in this mesially projecting mass, the identification of the placode is almost impossible. The relation of the general visceral IX to the placode is exactly like that in VII. The general visceral ganglion projects cephalad to the point where the placode forms, and ends in most series between 8.8 and 9.9 mm. just at the mesial tip of the placode; sometimes, however, it projects farther cephalad as it always does in series older than 9.9 mm. and its anterior end rests upon the ectodermic invagination.

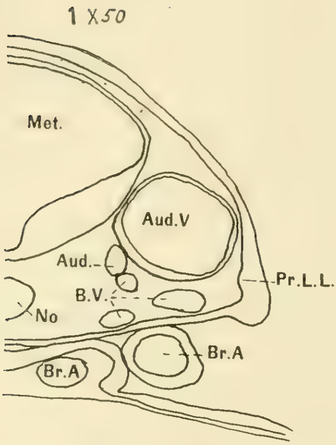
The difficulty found in the VII in regard to the relation of the sensory line to the placode does not exist here. The post-hyoid sensory line (dorso-lateral) extends beyond the anterior end of the IX ganglion but does not reach, as a definitely differentiated column of cells, the posterior end of the auditory vesicle. This sensory line lies dorsal to the placode and entirely detached from it so that there can be no doubt that the two structures are quite distinct and the condition here strengthens the opinion expressed in discussing these conditions in the VII, that the relation of the preauditory to the epibranchial placode was one of contiguity only and due to the fact that in the forward growth of the pre-auditory placode it occupies the same level as the epibranchial placode. This conclusion is still further strengthened from the study of Ameiurus (Landacre, '10) where the degeneration of

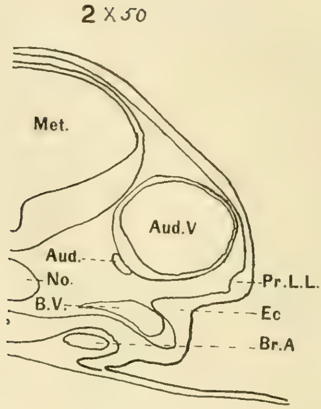
the anterior end of the preauditory placode could be followed preceding the appearance of the epibranchial placode. The relative position of the placode, general visceral IX, and ectodermic wall of the gill, are shown in text-figs. 1, 2, and 3 (p. 34).

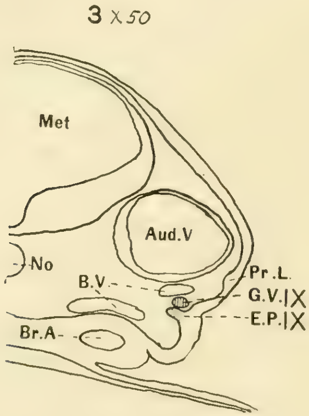
The first positive trace of the placodal cells was found in an 8.8 mm. embryo. This is shown in fig. 44. It differs somewhat from the early condition usually found, in that the ectodermic invagination of the gill pocket extends mesially to the placode and some cells projecting from the ectoderm in the region of the anterior end of visceral IX do not stain like placodal cells and are not genetically continuous with placodal cells. The reason for the greater extent of the ectodermic invagination in the IX, as well as in the first two branchial X, is the more acute angle at which the gill joins the roof of the pharynx.

The placodal cells are usually found immediately posterior to the ectodermic invagination of the gill pocket. However, there are ectodermic cells not belonging to the placode situated mesially to the placodal cells. The ectodermic gill invagination forms a curved mass reaching from in front of the placode to the mesial side of the placode of the IX. The placodal cells seem to have pushed their way into the posterior end of the ectodermic gill invagination. One section anterior to fig. 44 the placodal cells are absent and one section posterior to this point the section passes through into the general visceral ganglion which is barely in contact with the epidermis. It is thus seen that at this stage the placode in its anterior-posterior extent is quite thin, as are all the placodes in Lepidosteus. Between the 8.8 mm. and the 9.9 mm. embryo this mass of placodal cells cannot be identified always but in the 9.9 mm. embryo they become permanently established and their continuity can be traced up to the time they become detached from the ectoderm and incorporated into the general visceral IX.

In a 9.7 mm. embryo (fig. 45) we find the usual form of the placode. It consists of a mass of cells projecting mesially from the epidermis with the thickest portion of the placode lying ventral to the point of attachment. Mitotic figures are numerous in both the placode and the epidermis. On its anterior surface it







Text figures 1, 2 and 3 are camera tracings through the region of the IX gill from a 9.7 mm. embryo of Lepidosteus.

Fig. 1 passes through the point of contact of the first true gill with the roof of the pharynx.

Fig. 2 passes through the ectodermic gill shelf (*ec.*) seven sections posterior to fig. 1

Fig. 3 passes through the epibranchial placode of the IX and through the anterior end of the general visceral IX. The resemblance stucturally between the ectodermic gill shelf and the placode is striking. The placode can be recognized by its darker color.

Aud.V., auditory vesicle; *Aud.*, auditory ganglion; *B.V.* blood vessel; *Br.A*, branchial artery; *E.P.IX*, epibranchial placode of IX; *Ec.*, ectoderm of gill shelf; *G.V.IX*, general visceral ganglion of IX; *Met.*, Metencephalon; *No.*, notochord; *Pr.L.L.*, primordium of lateral line.

abuts against the ectodermic gill invagination and on its posterior surface it abuts against the anterior end of the general visceral IX. It is thus wedged in between the ectodermic gill invagination and the general visceral portion of the IX. From the 9.9 mm. stage on, the anterior end of the IX grows forward so that it rests upon the shelf formed by the ectodermic gill invagination. In some embryos younger than the 9.9 mm. stage, cells are found on this shelf but it is a constant feature of the ganglion from this stage on.

In the 10 mm. stage, the ganglion has assumed the comma shape (fig. 46) consisting of a rounded mesial portion and a lateral projection extending toward the epidermis and resting upon the extreme posterior end of the ectodermic gill invagination. This lateral projection is not continuous with the epidermis, but one section posterior to the one figured it is continuous. It is not possible at this stage to distinguish accurately by color differentiation between the placode and the ganglion cells presumably of neural crest origin. Since, however, the placode is not yet completely detached from the epidermis, one can trace the derivative of the placode by the form of the cells and their continuity with the epidermis.

After the detachment of the placode from the epidermis the placodal cells become, as in the VII, sharply differentiated in color from the remainder of the ganglion. The general form of the placodal mass is quite similar to that of VII, consisting of a central core more or less incorporated into the general visceral and projecting laterally and caudally (figs. 47, 48). This spur of cells is incorporated into the ganglion and cannot be detected in the 13 mm. stage, but is present in the 12.4 mm. stage. The remnant of the mass of placodal cells can be found in the 24 mm. stage and similar small dark staining cells can be found in the anterior tip of lateralis X indicating, as suggested in the discussion of the similar condition in the trigemino-facial complex, that there is a delayed development of these cells and that in Lepidosteus, if the cells retarded in development happen to be aggregated, the appearance described above is found, since it occurs in both the Gasserian and lateralis X and is always found in the pla-

codal ganglia. As in the case of the VII, the number of undifferentiated placodal cells is apparently greatly reduced in the later stages of my series and one can find cells lying at the periphery of the incorporated placodal cells and consequently in contact with general visceral cells, intermediate in size between the small and large size of ganglion cells. The late differentiation of these cells is apparently correlated with the late appearance of the ganglia and taste buds as stated above.

THE FIRST EPIBRANCHIAL PLACODE OF THE X

There are four epibranchial ganglia on the X nerve, as is usual among the Ichthyopsida. They are smaller than that on the VII and correspond closely in size and position to the IX. There is, however, owing to the differences in size and position of the general visceral ganglia, to which the special visceral ganglia are added, considerable variation in shape. The difference in position of the last gills alters also the appearance of the placode, particularly its relation to the ectodermic invagination.

Reference to fig. 1 will show that the first branchial ganglion of X resembles the IX in general form, being an elongated mass of cells placed nearly vertical in the body with its dorsal extension joining the large body of cells which constitutes the larger portion of general visceral X. This large mass of cells which contains the remaining three branchial ganglia of X is not so definitely formed into branchial ganglia as in Menidia. There is, however, at the point where each placode is formed a mass of cells projecting ventrally, to which the placodal cells are added, so that, while the presence of branchial divisions is indicated, the bulk of the general visceral cells is contained in one large general visceral ganglion.

The size of general visceral X and the fact that the branchial divisions are less marked give an appearance to the placodes that lends itself to the older interpretation of these structures, i.e., that they are epidermic thickenings with which the neural crest ganglia come into contact, but a careful examination of a series of embryos taken at close intervals shows that they resem-

ble closely the conditions already described for VII and IX nerves and consist of proliferations of cells derived from the ectoderm and added *en masse* to the neural crest ganglia.

The first placodal ganglion of the X appears in a 10 mm. stage (fig. 49). It projects mesially from the ectoderm at the extreme anterior end of the general visceral X, of which it forms the most anterior portion. No portion of the first branchial X lies upon the ectodermic shelf at this stage. It also abuts against the posterior end of the ectodermic gill shelf, from which it can be distinguished by its color and the arrangement of its cells as well as by the fact that it becomes detached later.

The relation of the epibranchial placode to the dorso-lateral sensory line is similar to the condition in the IX. The dorso-lateral sensory line lies somewhat dorsal to the placode and quite distinct from it. This placode is continued as in the IX posterior to the point of detachment by a thickened column of cells which disappears before reaching the next placode. Evidently the dorso-lateral sensory line and caudal prolongation of the placode are quite distinct structures in both IX and first branchial X and must be reckoned with in the case of the VII also if one is to interpret correctly the structures lying in the region of the first epibranchial placode.

In the 10.9 mm. embryo the placode is attached to the ectoderm by a slender cord of cells only, the lateral prolongation described in the VII and IX. This lateral prolongation extends backward also. In the 11.5 mm. stage (fig. 50) the placode is completely detached from the ectoderm, although both the placodal cells and the general visceral cells of the ganglion are in contact with the ectoderm; in fact, the ganglion rests upon the lateral roof of the pharynx. Before the placode is detached from the ectoderm the color differentiation between placodal and general visceral cells is marked and continues to be marked in all my series up to the 24 mm. stage without so apparent a reduction in the number of placodal cells as is shown in the VII and IX. The later differentiation of the placodal cells here and in the remaining placodes of the X is to be expected on account of their later appearance as compared with the VII.

THE SECOND EPIBRANCHIAL PLACODE OF THE X

The second epibranchial placode appears first in my series in a 10 mm. embryo (fig. 51). As in the case of the first epibranchial placode of the X, it lies at the anterior end of the corresponding general visceral branchial ganglion (fig. 1) and at the posterior end of the ectodermic shelf of the third true gill. It lies below the fundament of the lateral line and it is continued caudad in a thickening of the epidermis which does not reach the next gill and is throughout its course entirely distinct from the fundament of the lateral line. Fig. 52 shows the condition of the placodal portion of this ganglion in a 10.9 mm. embryo. It is completely detached from the epidermis laterally but still rests upon the epidermis and has both the lateral and caudal prolongations. The later history of this placode parallels those already described and the placodal cells can be readily detected in my oldest series (24 mm.)

THE THIRD AND FOURTH EPIBRANCHIAL PLACODES OF THE X

The third epibranchial placode of the X appears first in my series in a 10.9 mm. embryo (fig. 53). It differs from those already described in being less pronounced both as to size and color differentiation. In some stages succeeding the 10.9 mm. the placodal cells are difficult to detect, although they can always be found if the approximate location of the placodal cells is determined with reference to the corresponding gill bar.

The appearance of this placode after detachment from the ectoderm and before complete incorporation into the general visceral ganglion and while still possessing the lateral and caudal spurs, is shown in fig. 54. The fundament of the lateral line is not present at the level at which this section is taken, owing apparently to the absence of a lateral line organ at this point, but slightly posterior to this point it is present and occupies a level above the epibranchial placode. The thickening of the epidermis running caudad from the point of origin of the epibranchial placode is not pronounced but is present. The later history of the placode, including its incorporation into the general vis-

ceral ganglion, offers no new features. It can be detected in the 24 mm. stage.

The fourth epibranchial placode of the X appears first in a 12.9 mm. embryo (fig. 55). The posterior surface of the fourth gill bar of the X does not become detached from the ectoderm but this does not alter the relations greatly. There is no ectodermic shelf and I can detect no thickening of the epidermis extending caudad from the point of origin of the epibranchial placode. The fundament of the lateral line in this region lies far dorsal at the level of the lateral line nerve of the X. This nerve lies on a level with the middle of the notochord. The later history of this placode duplicates the history of the preceding placodes described. The lateral and caudal prolongations are less pronounced but it becomes incorporated into the last general visceral branchial ganglion and can be located in the 24 mm. series.

GENERAL SUMMARY AND DISCUSSION

1. The epibranchial placodes of Lepidosteus osseus arise as proliferations of the ectoderm at the dorsal and caudal border of the corresponding gill bar. They project mesially and finally become detached *en masse* and fuse with the general visceral portions of the VII, IX and four branchial ganglia of the X nerve. The point on the general visceral ganglion at which the placodal cells join it is always near its anterior end, sometimes in such a manner as to form the extreme anterior tip of the corresponding ganglion, sometimes, however, joining it laterally and ventrally in such a manner as to be partly surrounded by the general visceral cells.

2. Preceding the detachment of the epibranchial placodes their history can be followed, owing to their continuity with the ectoderm, from which they differ both in color intensity and in histological character. In the earlier stages, however, especially in the case of the VII nerve whose epibranchial placode is by far the largest and most conspicuous of the series, difficulties arise because of the presence of other thickenings in the ectoderm, i.e., (a) the primordia of the lateral sensory lines, (b) the thickening

of the epidermis at the point where the pharyngeal endodermic pocket joins the ectoderm, (c) the anterior extension of the auditory vesicle (the preauditory placode of Ameiurus, Landacre, '10, the branchial sense organ of Wilson, '91), (d) the thickening of the epidermis extending caudad from the point of origin of the epibranchial placode.

In all the epibranchial placodes there is a thickening of the epidermis at the point where the endodermic gill pocket joins the ectoderm and this always lies anterior to the point of origin of the placode. The epibranchial placode always arises at the posterior end of this thickening and while in Lepidosteus the placodes are unusually long in their transverse axis they are thin from anterior to posterior and abut against the posterior border of the ectodermic gill invagination. In all the placodes except the VII, and sometimes here, there is a sharp color differentiation as well as histological differentiation between the two structures and one can be quite sure of this distinction in the later stages of the placode, although the two structures are continuous, when one reads the sections from the ectodermic gill shelf into the placode.

The primordia of the lateral sensory lines can be differentiated from the epibranchial placodes with equal ease except in the case of the VII. They lie at a different level, much above the placodes in IX and X, but in VII the supra-orbital, sub-orbital and mandibular sensory lines converge at the hyoid gill rendering it difficult to differentiate between primordia of lateral sensory lines and the early stage of the epibranchial placode. Much the same difficulty exists in the VII with reference to the preauditory placode. This structure in its anterior extension drops to the level of the hyoid gill and seems in the earlier stages, before the appearance of the epibranchial placode, to become continuous with the ectodermic gill thickening in this region although it changes its histological characters at the anterior end. To add to these difficulties in the case of the VII, each epibranchial placode in Lepidosteus is continued caudad by a thickening of the epidermis, which in the case of the IX and X ganglia lies at a lower level than the fundament of the lateral lines and of course in these cases cannot be confused with the dorso-lateral placodes.

In the VII the column of cells extending caudad from the epibranchial placode and persisting after the detachment of the placode occupies the same level in the epidermis as that previously occupied by the preauditory placode. There can be no doubt, however, from an examination of the epibranchial placodes of IX and X that this is a different thickening from either the preauditory placode or the primordia of the dorso-lateral sensory lines. I interpret all these as distinct structures, including the epibranchial placode and its posterior extension in the epidermis, the preauditory placodes and primordia of the sensory lines and the ectodermic thickening at the point where the endoderm of the hyoid gills joins the ectoderm, on evidence furnished by the study of similar structures in the IX and X ganglia; and I conclude that they are simply contiguous in the VII. As to the exact relation of the sensory lines to the preauditory placode, there is some degree of uncertainty in view of the conflicting evidence furnished by Wilson ('91) on Serranus and my own work on Ameiurus.

The significance of the posterior extensions of the epibranchial placodes is problematical also. They are certainly not closely related to the fundament of the lateral lines in the IX and X, being, in fact, entirely distinct from them. The whole situation emphasizes the extreme caution that must be exercised in making statements in regard to the relations of sensory lines lying in the region of the VII either epibranchial or dorso-lateral, and in particular in regard to the relation of the preauditory placodes to the supra-orbital, sub-orbital and mandibular sensory lines, since the point at which the preauditory placode is supposed to split up into sensory lines is in the region of the epibranchial placode of the VII. The hyoid gill region becomes the focal point in the differentiation of all these structures. The easiest of all these structures to follow is the epibranchial placode of the VII after it once becomes established, although it is difficult to locate in its early stages. The growth of all the placodes, including the VII, can be followed with ease to the time when they reach their maximum size. Both their structural and color differences are sharp. The placodes take a darker stain than either

the epidermis or the general visceral ganglia and their cell arrangement is characteristic, the cells being closely packed with numerous mitotic figures. The body of the placode is apparently derived from the deeper nervous layer of the ectoderm ventral to the point of attachment. The ectoderm dorsal to the point of attachment of the placode is always thin.

3. During the time of detachment and throughout their later history the placodal cells can be followed for the same reasons; in fact, the placodal cells during their later history and up to the time they become metamorphosed into ordinary ganglion cells are more sharply differentiated from general visceral cells than in their earlier stages and present a striking feature in the ganglia of which they are components. The ease with which these placodal cells can be followed in Lepidosteus seems to be unique among the Ichthyopsida so far studied.

Immediately after the epibranchial placodes become detached from the epidermis and during the earlier stages of their incorporation into the general visceral ganglia they occupy the ventral or ventro-lateral portion of the corresponding ganglion and always have a spur of cells projecting laterally toward the epidermis at the point at which the placode became detached. This spur of cells always except in the case of the fourth branchial ganglion of the X projects caudally also.

In the later history of each ganglion this spur becomes incorporated into the larger mass of placodal cells which at first, while being largely surrounded by general visceral cells, always reaches the external boundary of the ganglion at its ventral surface. Still later in the history of each ganglion the placodal cells are found completely surrounded by general visceral cells or cells of the same type. At the boundary between the incorporated placodal cells and the surrounding general visceral cells there are found in embryos of 12.4 to 24 mm., especially in the VII ganglion, cells varying in size from that of the minute dark staining placodal cells to that of the ordinary visceral ganglion cells. This indicates that the placodal cells are gradually transformed into ordinary ganglion cells indistinguishable from general visceral ganglion cells. Some of these small dark staining cells persist

in the oldest of my series and there are such cells found in a 44 mm. embryo and even in a 152 mm. fish at approximately the point where the placodal cells should be, although my series are not continuous up to these older stages.

The fact that the history of the placodal cells can be followed so definitely seems to depend upon two things; first, the late appearance of the placodal ganglia as compared with the general visceral ganglia and the retarded development of the gustatory organs of Lepidosteus as compared with such a form as Ameiurus. This sets the immature placodal ganglion off in sharp contrast with the older and more mature general visceral ganglia. As the placodal cells become transformed into normal ganglion cells, they can no longer be distinguished from the general visceral cells.

In the second place, the identification of the placodal cells seems to depend upon the fact that in the ganglia of Lepidosteus the histological distinction between immature ganglion cells and mature ganglion cells is unusually sharp, so that if the immature ganglion cells happen to be aggregated they become, owing to their small size and dark staining properties, quite conspicuous. Such masses of cells can be found in a 24 mm. embryo in both the Gasserian and in the lateralis X ganglion but not in the earlier stages of these ganglia. Since there are no epibranchial placodes on these ganglia, these masses of cells are to be interpreted as immature cells. Such immature cells are usually found in ganglia but become especially prominent when collected in definite masses. The nerve fibers arising from ganglia containing both placodal cells and neural crest cells are not sufficiently different from each other in the oldest specimen examined (152 mm.) to enable one to trace the gustatory and general visceral fibers to their respective ganglion cells and peripheral terminations, so that for the present the reason for the classification of the placodal cells as special visceral cells must rest on the evidence offered in a later paragraph.

4. The explanation suggested in the body of the paper, that the observed difference between the placodal cells and the remaining cells of the general visceral ganglia of the VII, IX, and X nerves is due to the relatively late appearance of the gustatory

organs, finds confirmation in a comparison of the relative time of appearance of the placodes and taste buds in Lepidosteus and Ameiurus (Landacre, '07).

Approximately the same time, five days, intervenes between the fertilization of the eggs and the time of hatching in Lepidosteus and Ameiurus. If we compare the size at any given age, and rate of growth for any given period, Lepidosteus is found not only to be longer but to grow faster than Ameiurus. At the age of 113 hours Ameiurus is 5.73 mm. long, while at the nearest corresponding age, 112 hours, Lepidosteus is 8 mm. long. Between the ages of 113 hours and 199 hours, Ameiurus increases 2.77 mm. in length, while Lepidosteus between the ages of 112 hours and 196, my nearest corresponding series, increases in length 5 mm.—a total difference in growth of 2.23 mm. in favor of Lepidosteus. During this period Ameiurus increases 43.3 per cent while Lepidosteus increases 62.5 per cent.

If now we compare the more rapid rate of growth in Lepidosteus with the time of appearance of preauditory placodes, the length of time intervening between the appearance of the first placode and the appearance of the last, the time at which taste buds first appear, and lastly the total number of taste buds at any given age, we shall find all these processes beginning earlier and being completed earlier in Ameiurus, which is the slower growing form.

The first epibranchial placode appears in Ameiurus preceding the 49-hour stage and the last placode appears at 105 hours. In Lepidosteus the first placode appears at 94 hours and the last at 191 hours. There is a difference of 45 hours between the time of appearance of these structures and a difference of 41 hours in the time intervening between the appearance of the first and the last placode, the placodes appearing earlier and consuming less time in their formation in Ameiurus which measured by length grows slower than Lepidosteus. The first taste buds appear in Lepidosteus at 191 hours, 97 hours after the appearance of the first placode while in Ameiurus the first taste buds appear at 113 hours, 74 hours after the appearance of the first placode. So that here again the taste buds appear later and more time

intervenes between the appearance of the placode and the appearance of the taste buds in Lepidosteus than in Ameiurus, which is the slower growing form. Of more significance still, is the relative number of taste buds present at a stage when both types have them developed fully enough so that they can be counted with certainty. Two series fulfil these conditions closely, Lepidosteus at 214 hours and Ameiurus at 213. At the age of 214 hours, Lepidosteus has 32 taste buds distributed on the roof of the oral cavity and pharynx, and on all five gills. Owing to the large number of mucous glands on the outer surface of the body and the presence of the adhesive disc on the head where external buds are most likely to be found, it is not possible to determine the number in the skin.

In Ameiurus at 213 hours there are 146 taste buds in the oral cavity, 352 in the pharynx and gills and 117 situated externally on the skin of the head chiefly. This gives a total of 615 taste buds in Ameiurus as compared with 32 for Lepidosteus for a corresponding age. The later appearance and slower rate of growth of ganglia and limited number of taste buds in Lepidosteus as compared with Ameiurus of the same age seem to offer, when we consider the different rate of growth in the two types, strong evidence that the gustatory system of Lepidosteus is retarded in development as compared with other structures in this form and that this retardation is indicated in the histological differences between placodal and neural crest cells and offers a satisfactory explanation of the retarded metamorphosis of the special visceral cells into normal ganglion cells.

5. The assumption that cells derived from the epibranchial placodes, after their metamorphosis into ganglion cells, are the cells giving rise to fibers supplying taste buds and consequently designated as special visceral ganglia, rests upon indirect evidence but evidence of such a character as to warrant the assumption in view of the extreme difficulty of demonstrating its truth or falsity. Epibranchial placodes are found in all types of vertebrates, even among mammals, including man. Among the lower vertebrates where their history can be followed in series taken at close intervals, they can be shown in some cases to con-

tribute cells to the neural crest portions of the corresponding ganglia. In other types where the placodes are less prominent an actual contribution of cells to the neural crest portion of the corresponding ganglia has not been shown to take place, but a contact is formed between the neural crest ganglia and the epidermis in all cases. In view of the reduced character of the gustatory system in many forms as compared with the fishes, it is not surprising that the actual contribution of cells by the epibranchial placode should not be large and might take place during the period of contact and still be difficult to demonstrate. There seems to be no other adequate explanation for the formation of this contact between the neural crest ganglia and the ectoderm in such types that would harmonize with the known conditions in Ameiurus and Lepidosteus.

In all types epibranchial placodes occur on those nerves and those only, VII, IX and X, which contain gustatory fibers. Whenever, as is usually the case among the Ichthyopsida, the ganglia of these nerves are complex, containing lateralis, cutaneous and visceral components, the placodal cells join only the visceral ganglia, never the lateralis or general cutaneous ganglia. These visceral ganglia give rise to fibers supplying general visceral surfaces and taste buds only. In Ameiurus and Lepidosteus the various components of the ganglia are so distinct in the embryos that this conclusion can not be doubted and the problem resolves itself into the effort to differentiate special visceral or gustatory, and general visceral ganglia. Ganglia such as the profundus and Gasserian which do not contain visceral fibers of either type and have no epibranchial placodes, can be eliminated. With equal certainty we can eliminate all lateralis and general cutaneous ganglia in VII, IX and X, since they give rise to no visceral fibers of either type and have no epibranchial placodes.

An examination of the visceral ganglia of Ameiurus shows that the VII ganglion, which in the adult supplies a large number of taste buds, has a large placode, while the last branchial ganglion of the X which supplies in most types a limited number of taste buds, has a small placode. The visceral portion of the IX seems to be exclusively placodal in origin and seems in the adult to give

rise to gustatory fibers only. So that in general there is a close correspondence between the size of the epibranchial placode and the number of gustatory fibers to which the ganglion gives rise in the adult.

A study of Lepidosteus, in addition to furnishing a confirmation of the conclusion reached from a study of Ameiurus, shows in addition that the placodal ganglia maintain their integrity for a long time, although embedded in the general visceral ganglia, and that the late appearance of the epibranchial placodes and their slow metamorphosis into ganglion cells is closely correlated with the retarded appearance of the gustatory organs. This correlation, when taken in conjunction with the fact that only those ganglia having placodal cells give rise to gustatory fibers and in proportion to the size of the placodes and that all ganglia having epibranchial placodes give rise to gustatory fibers and particularly that a ganglion that is apparently exclusively placodal is also apparently exclusively gustatory, seems to warrant the assumption that placodal ganglia are special visceral ganglia.

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ABBREVIATIONS

Aud. V., Auditory vesicle.
Aud., Auditory ganglion
Br. IX, Branchial nerve of the IXth
Br. X_1, First branchial nerve of the Xth
Br. X_2, Second branchial nerve of the Xth
Br. X_3, Third branchial nerve of the Xth
Br. X_4, Fourth branchial nerve of the Xth
Br. A, Branchial artery
Bv., Blood vessel
Bu., Ramus buccalis VII
D. L. VII, Dorso-lateral ganglion of the VII
Ec., Ectoderm
En., Endoderm
Epi., Epiphysis
E. P., Epibranchial placode (posterior extension)
E. P. VII, Epibranchial placode of the VII
E. P. IX., Epibranchial placode of the IX
E. P. X_1, First epibranchial placode of the X
E. P. X_2, Second epibranchial placode of the X

E. P. X_3, Third epibranchial placode of the X
E. P. X_4, Fourth epibranchial placode of the X
Gass., Gasserian ganglion
Gen., Geniculate ganglion, general and special visceral
G. Cil., Ciliary ganglion.
G. IX, Glossopharyngeus ganglion
G. V. VII, General visceral portion of the VII
G. V. IX, General visceral portion of the IX
G. V. X, General visceral portion of the X
G. V. X_1, General visceral portion of 1st branchial of X
G. V. X_2, General visceral portion of 2nd branchial of X
G. V. X_3, General visceral portion of 3rd branchial portion of X
G. V. X_4, General visceral portion of 4th branchial of X
Hyo., Hyomandibular cartilage
Hyo. VII, Truncus hyomandibularis
L. IX, Lateralis ganglion of the IX
L. X., Lateralis ganglion of the X
Mand. V, Truncus mandibularis V
Max. V, Truncus maxillaris V
Met., Metencephalon
Mes., Mesencephalon
N. III, Oculomotor nerve
No., Notochord
O. VII, Ramus oticus VII
Olf., Olfactory capsule
Opt., Optic vesicle
O. Pro., Opthalmicus profundus nerve
O. S. V., Ramus opthalmicus superficialis V
O. S. VII, Ramus ophthalmicus superficialis VII
Pros., Prosencephalon
Pro., Profundus ganglion
Pal. VII, Ramus palatinus VII
Pr. L. L., Primordia of lateral lines
R. Aud., Root of auditory nerve
R. VII, Root of facialis
R. V, Root of trigeminus
R. L. VII, Root of lateralis VII
R. L. X, Root of lateralis X
*R. V. I, VI*Root of visceralis VII
R. V. X, Root of visceralis X
St. IX, Ramus supratemporalis IX
St. X, Ramus supratemporalis X
Sup. L, Supra-orbital lateral line
Sub. L, Sub-orbital lateral line
S. V. VII, Special visceral ganglion of VII
S. V. IX, Special visceral ganglion of IX
S. V. X, Special visceral ganglion of X
S. V. X_1, Special visceral portion of 1st branchial of X
S. V. X_2, Special visceral portion of 2nd branchial of X
S. V. X_3, Special visceral portion of 3rd branchial of X
S. V. X_4, Special visceral portion of 4th branchial of X
T. L. X, Truncus lateralis X

PLATE 1

A flat reconstruction of the brain, sense organs and sensory components of V, VII, VIII, IX and X cerebral ganglia of a 10 mm. embryo of Lepidosteus osseus.

General cutaneous ganglia are indicated by horizontal shading and this component is found at this stage in the profundus (trigeminus I) and the Gasserian ganglion (trigeminus II). The jugular ganglion of the X is not present at this stage; when it becomes organized it lies on the root of the X near the entrance of the root into the brain.

The special cutaneous ganglia are indicated by cross hatching and are found in the VII (dorso-lateral and ventro-lateral lateralis ganglia of the VII), in the VIII, IX and X.

General visceral ganglia are indicated by vertical lines and are found in the VII (geniculate), IX (petrosal), and X (nodosal).

The special visceral ganglia are indicated by stipple and are found in the VII, IX and X, there being only two of the four epibranchial ganglia of the X found at this stage. Aside from the hyomandibular nerve, all the nerve trunks are pure, i.e., contain only one component. The absence of the last two branchial ganglia and the lack of fusion in nerve trunks, which takes place later, are the chief differences between this embryo and older stages.

The scale at the side of the figure indicates the serial numbers of the sections from which the reconstruction was made.

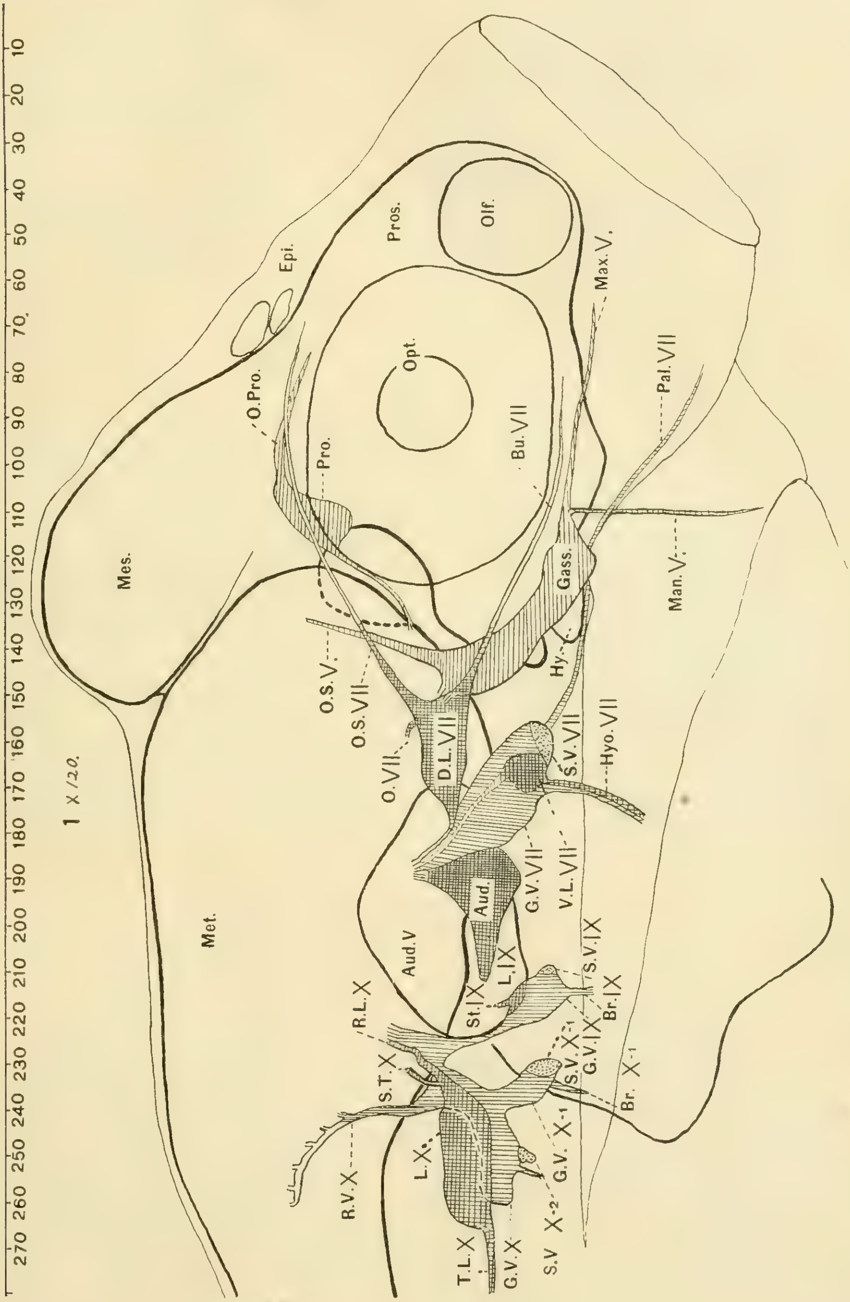

1 X 20.
Mes.
Met.
Epi.
Pros.
Olf.
Opt.
O.Pro.
Pro.
Bu.VII
Max.V.
Pal.VII
Man.V.
Gass.
Hy.
O.S.V.
O.S.VII
O.VII.
D.L.VII
S.V.VII
Hyo.VII
G.V.VII
V.L.VII
Aud.
Aud.V
L.IX
St.IX
S.V.IX
Br.IX
G.V.IX
R.L.X
S.T.X
S.V.X-1
G.V.X-1
Br.X-1
L.X.
R.V.X
G.V.X-2
S.V X-2
T.L.X
G.V.X

PLATE 2

A series of camera tracings of transverse sections of the head of Lepidosteus of 10 mm. length. The numbers following the number of the section indicate the level at which the section falls, as indicated on the scale over fig. 1. The sections are slightly diagonal, the right side of figure being further anterior.

Fig. 2 is taken at the level of the olfactory capsule and nerve.

Fig. 3 passes through the anterior portion of the optic vesicle and the epiphysis and dorsal sac.

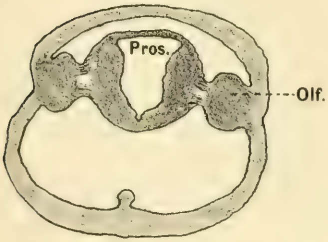
Fig. 4 lies just posterior to the lens and passes through the anterior end of the profundus ganglion.

Fig. 5 passes through the third nerve, on right side of figure, and the posterior end of the profundus ganglion. The anterior end of the hypophysis is cut in this section.

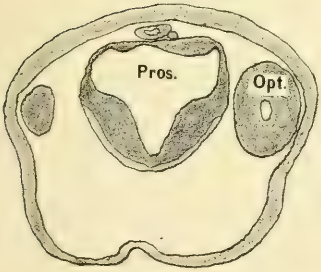
Fig. 6 passes through the posterior end of the Gasserian on right side of figure and the anterior end of the lateral lobes of the medulla.

Fig. 7 passes through the root of the Gasserian ganglion on right side and the posterior end of the mesencephalon.

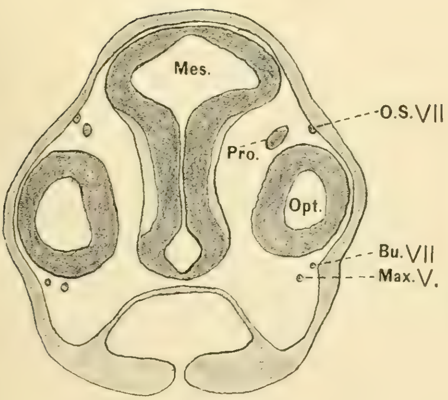
2=49.X50



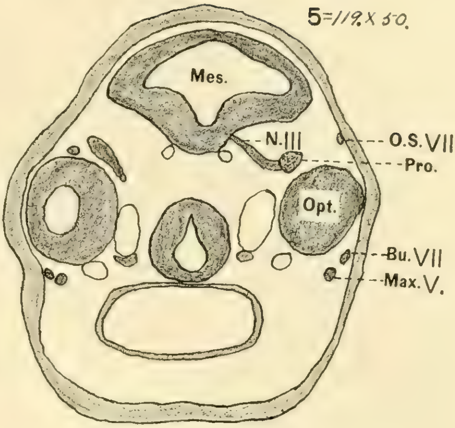
3=70.X50.



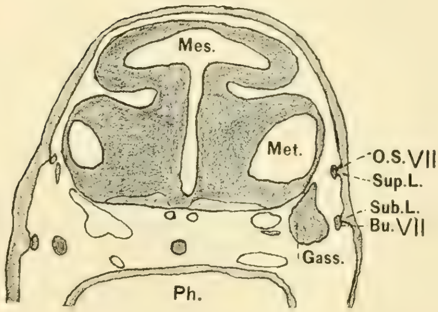
4=109.X50.



5=119.X50.



6=144.X50.



7=149.X50.

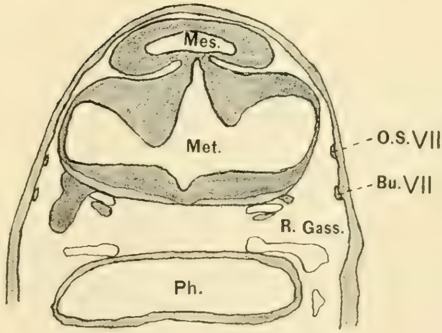


PLATE 3

A continuation of figures of plate 2. All references are to the right side of figures.

Fig. 8 passes through middle of dorso-lateral VII and through the geniculate ganglion at the point where the placode was attached to the epidermis. The area blocked out in this figure lies in the middle of the series of figures on succeeding plate (fig. 18).

Fig. 9 passes through the posterior end of dorso-lateral VII and through the middle of ventro-lateral VII. The geniculate is cut posterior to the placodal ganglion.

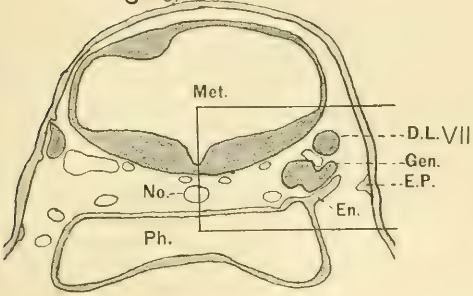
Fig. 10 passes through the middle region of the auditory capsule and through the IX ganglion at the point of origin of its visceral trunk.

Fig. 11 passes through the epibranchial placode of the first branchial ganglion of X and the root of the lateralis X ganglion.

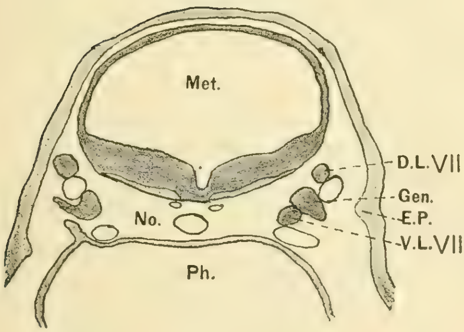
Fig. 12 passes through the middle of the lateralis X ganglion and through the visceral X just anterior to the epibranchial placode of the second branchial ganglion of X. The visceral root of X is cut in this section.

Fig. 13 passes through the posterior end of the lateralis X ganglion posterior to the posterior end of visceral X. The visceral root of X is cut in this section quite close to the medulla.

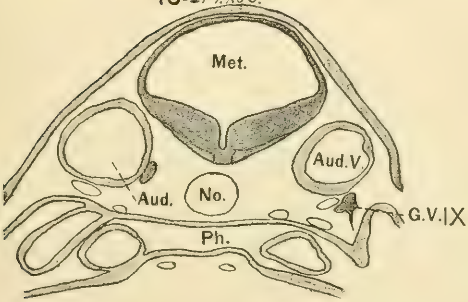
8=161.X50.



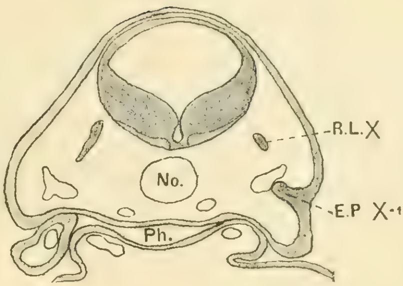
9=166.X50.



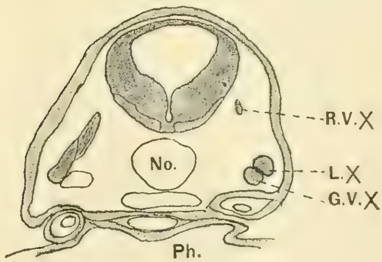
10=214.X50.



11=230.X50.



12=244X50.



13=261.X50.

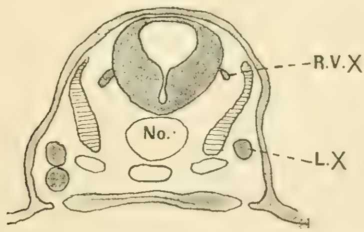


PLATE 4

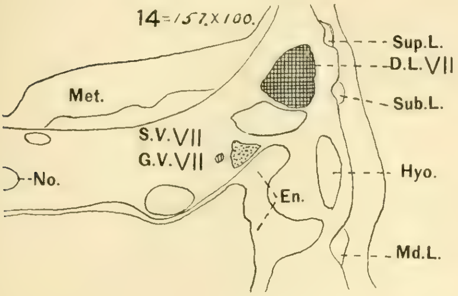
Figs. 14 to 21 are a series of camera tracings of transverse sections of the 10 mm. embryo extending from the anterior end of the geniculate ganglion to the posterior end of the placodal portion of this ganglion. The anterior third of the ventro-lateral VII is included in the last two figures (20 and 21). Lateralis components are indicated by cross hatching, general visceral by vertical lines, and special visceral by stipple.

Fig. 18 corresponds to the area blocked out in fig. 8 of the preceding plate.

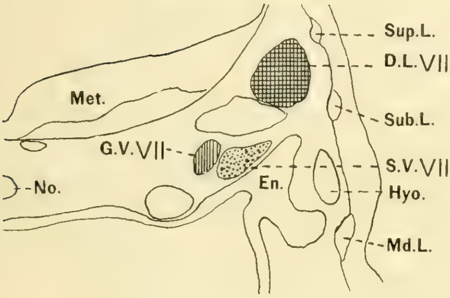
These figures were sketched from the same series as that from which the first reconstruction was made, fig. 1, and numbers of sections indicate position on scale of fig. 1. The figures are from consecutive sections. These figures show clearly the part the placode plays in the composition of the geniculate ganglion after the placode has become detached from the ectoderm.

Fig. 14 lies at the extreme anterior tip of the geniculate ganglion. See fig. 26 for detail of fig. 19, and fig. 27 for detail two sections posterior to fig. 21.

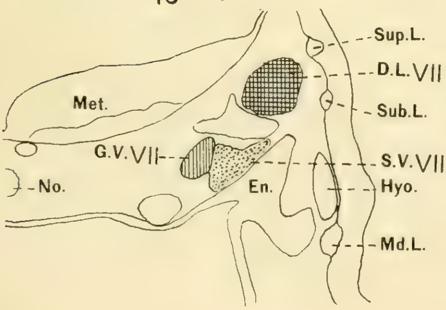
14=157.x100.



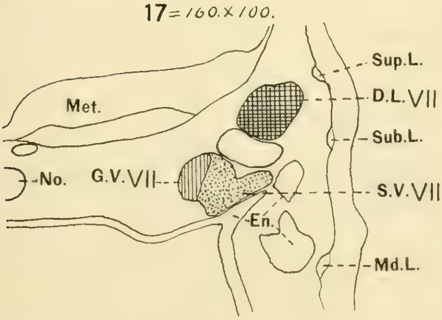
15=158.x100.



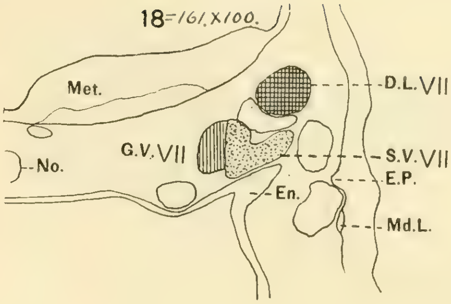
16=159.x100.



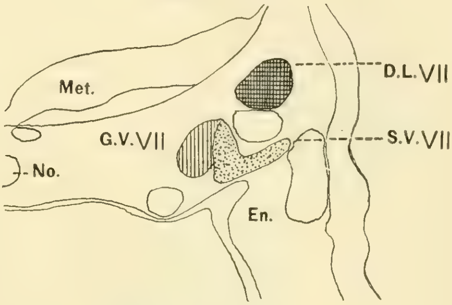
17=160.x100.



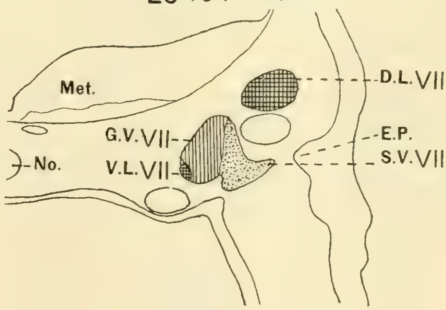
18=161.x100.



19=162.x100.



20=163.x100.



21=164.x100.

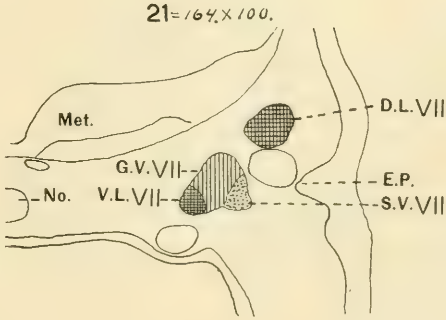


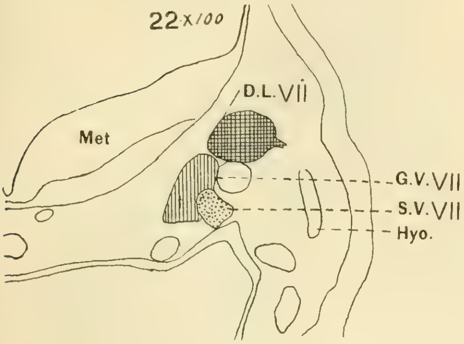
PLATE 5

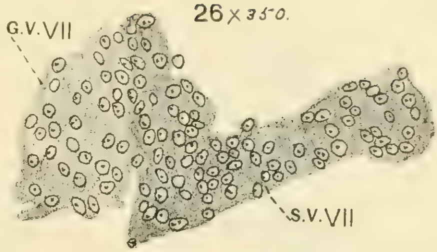
Figs. 22 to 25 from a 10.9 mm. embryo, are camera tracings to show the character of the placodal spur of the VII projecting laterally and caudally. Components are indicated as in preceding plate and in fig. 1. Lateralis or special cutaneous cross hatched; general visceral vertical lines; placodal or special visceral, stipple; figs 23, 24 and 25 are from consecutive sections. Two sections lie between figs. 22 and 23. The portion of this ganglionic complex shown in stipple is that which can be positively identified as placodal in origin. That more of the geniculate or general visceral should be shown as derived from the placode is possible, if the placodal cells have metamorphosed into ganglion cells. See fig. 28 for detail of fig. 23.

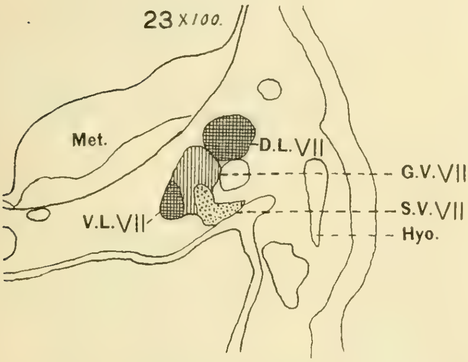
Fig. 26 shows the histological detail of the general visceral and placodal ganglia in fig. 19. The color differentiation due to size and density of cells is well shown in this figure. Length of embryo 10 mm.

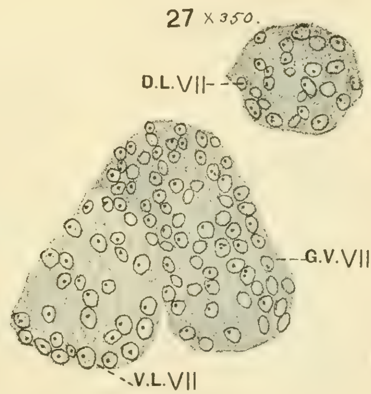
Fig. 27 shows the detail of dorso-lateral, ventro-lateral and geniculate ganglia posterior to the placodal portion in a 10 mm. embryo. This section lies two sections posterior to that shown in fig. 21.

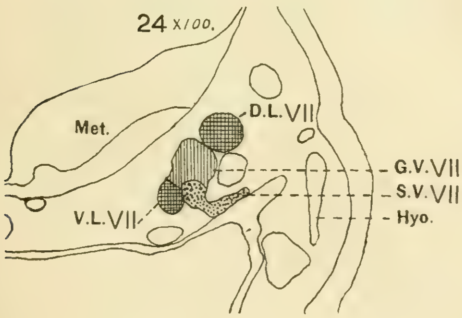
Fig. 28 shows the detail of the geniculate ganglion and the ventro-lateral ganglion in a 10.9 mm. embryo. This figure gives the histological detail of fig. 23 minus the dorso-lateral VII. Fig. 28 should be compared with fig. 26, since they are taken through approximately the same region of the geniculate ganglion of embryos differing nearly 1 mm. in length. The constancy in appearance of the placodal ganglion is striking.

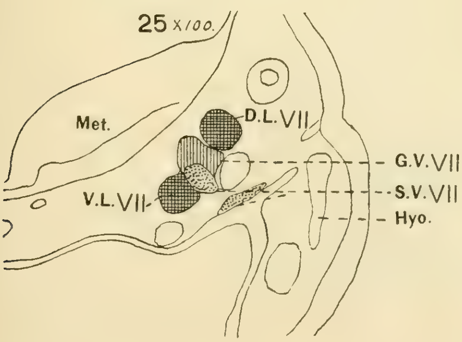

22 ×100
Met
D.L.VII
G.V.VII
S.V.VII
Hyo.


26 ×350.
G.V.VII
S.V.VII


23 ×100.
Met.
D.L.VII
G.V.VII
S.V.VII
V.L.VII
Hyo.


27 ×350.
D.L.VII
G.V.VII
V.L.VII


24 ×100.
Met.
D.L.VII
G.V.VII
S.V.VII
V.L.VII
Hyo.


25 ×100.
Met.
D.L.VII
G.V.VII
V.L.VII
S.V.VII
Hyo.

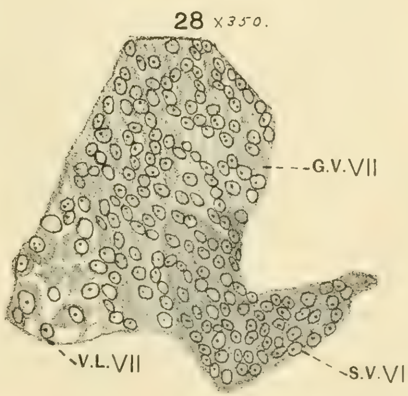

28 ×350.
G.V.VII
V.L.VII
S.V.VII

PLATE 6

Fig. 29 from a 12.4 mm. embryo, is taken from approximately the same position in the VII ganglionic complex as fig. 25, which is from a 10.9 mm. embryo. There are no placodal cells present in this section except the caudally projecting spur. This is soon incorporated into the geniculate ganglion. The close resemblance histologically between the dorso-lateral and the geniculate, and the distinct character of the ventro-lateral which are so noticeable in this figure, can be detected in all earlier stages.

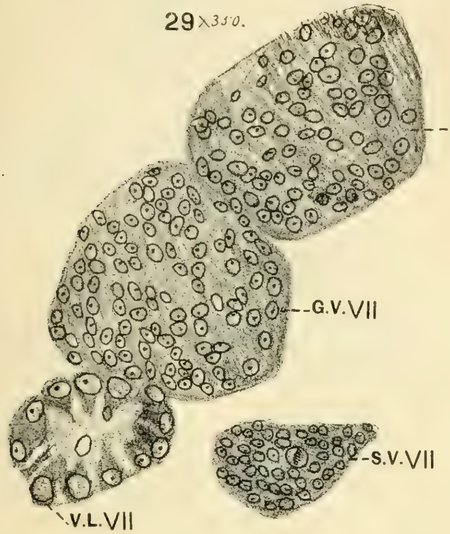
Fig. 30 is taken from the same embryo (12.4 mm.) as that from which fig. 29 was taken and lies five sections anterior to fig. 29. Only the anterior tip of the ventro-lateral ganglion shows and the dorso-lateral has been omitted on account of its size. The placodal ganglion projects somewhat from the ventro-lateral surface of the geniculate.

Fig. 31 shows the same relations in a 13.5 mm. embryo. The placodal ganglion is almost completely incorporated into the geniculate. The distinction shown here between unaltered placodal cells and the normal ganglion cells of the geniculate is fairly representative for all later stages in which placodal cells can be detected.

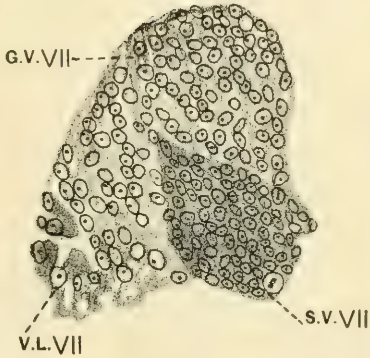
Fig. 32 shows the detail of the epibranchial placode of the VII in a 9.5 mm. embryo and illustrates the relation of the placode to the ectoderm above and below its point of attachment. This figure corresponds to the middle of the placode shown in fig. 33.

Fig. 33 is a sketch of a wax reconstruction of the epibranchial placode of VII and associated structures in the ectoderm and endoderm. The model rests on its anterior surface and is seen from the ventral surface. The ectoderm with its attached epibranchial placode is detached from the endoderm and that portion of the ectoderm lying anterior to the placode. The hyoid gill pocket lies to the right and is closely fused with the ectoderm back to the point of origin of the placode. If the two portions of the model are placed together it will be seen that the anterior surface of the epibranchial placode rests upon the posterior surface of that portion of the hyoid gill pocket that comes into contact with the ectoderm. The length of the embryo from which the model was reconstructed was 9.7 mm.

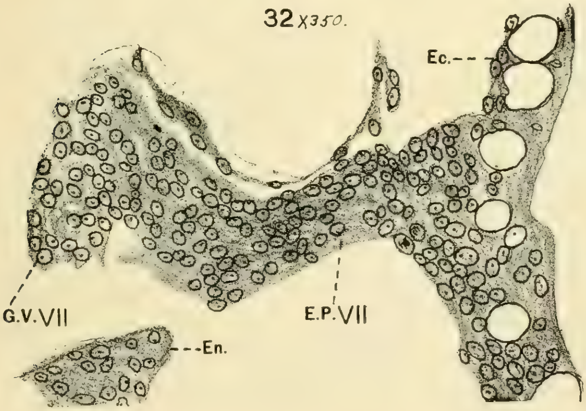
29 ×350.



30 ×350.



32 ×350.



31 ×350.

33

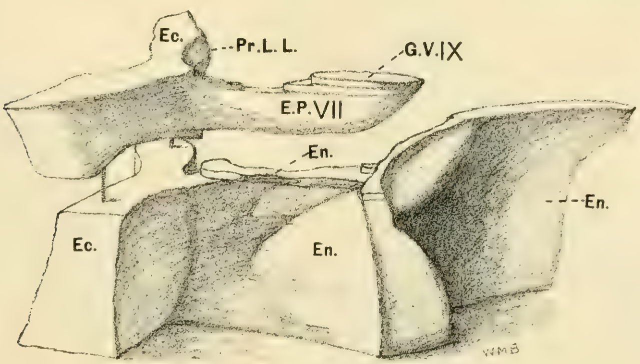


PLATE 7

Fig. 34 from an 82 hr. embryo is taken through the posterior portion of the thickening of the ectoderm at the point where the endoderm of the pharyngeal gill pocket joins it. The ectoderm is easily distinguished by its color.

Fig. 35 from an 82 hr. embryo is taken through the thickening in the epidermis just posterior to the hyoid gill pocket and is presumably the anterior extension of the preauditory placode and, although it is not detached from the auditory vesicle, it shows none of the histological characters of the preauditory placode. Fig. 35 lies three sections posterior to fig. 34.

Fig. 36 from a 94 hr. embryo, is taken at the extreme posterior limit of the contact of the pharyngeal gill pocket with the ectoderm. The nuclei of the ectoderm are closely packed and the cell walls are indefinite or absent, showing distinctly that it is related to the epibranchial placode rather than to the preauditory placode.

Fig. 37 from a 94 hr. embryo is taken just posterior to the contact of the hyoid gill pocket with the ectoderm and shows the proliferation of cells to form the epibranchial placode. Fig. 37 is the next section posterior to 36.

Figs. 36 and 37 show the absence of any primordium of the lateral lines in the region of the placode up to this age.

Fig. 38 from a 7.3 mm. embryo is taken at the posterior limit of the contact of the hyoid gill pocket with the endoderm. In addition to the ectodermic gill thickening there is a dorsal thickening (primordium of lateral line).

Fig. 39 from a 7.3 mm. embryo is taken just posterior (two sections back of fig. 38) to the contact of the hyoid gill pocket with the ectoderm. It shows three thickenings; most dorsal, the primordium of the lateral line; most ventral the probable remnant of the preauditory placode. The middle thickening is the epibranchial.


34 X350.
Ec.
En.
35 X350.
Ec.
36 X350.
Ec.
E.P.VII
En.
37 X350.
Ec.
E.P.VII
En.
38 X350.
Pr L. L.
Ec.
En.
39 X350.
Pr L. L.
Ec.
E.P.VII
En.

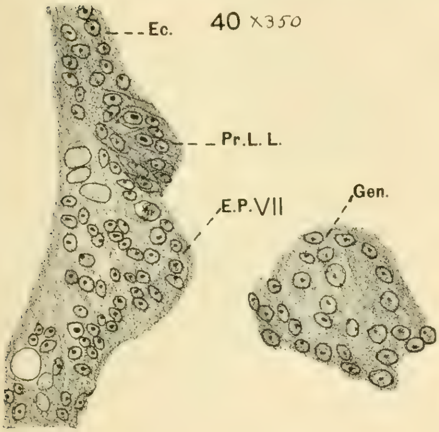
PLATE 8

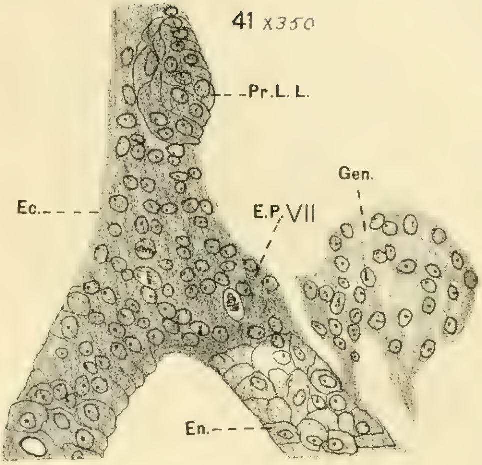
Fig. 40 is from a 7.3 mm. embryo. The section passes through the posterior extension of the epibranchial placode of VII and the posterior extension of the primordium of the sensory line lying dorsal to it. This section contains the anterior end of the geniculate ganglion and lies four sections posterior to fig. 39.

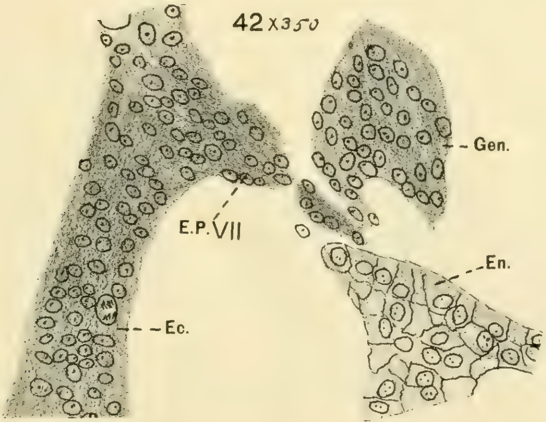
Fig. 41 from an 8 mm. embryo, passes through the extreme posterior portion of the contact of hyoid gill pocket with the ectoderm. It shows in addition to the differentiation between ectoderm and endoderm the fact that the geniculate ganglion sometimes extends forward over the phyarngeal gill pocket. The primordium of the sensory line lies well dorsal to the ectodermic gill thickening.

Fig. 42 from an 8 mm. embryo, shows the first section posterior to the point where the hyoid gill pocket withdraws from the ectoderm. The epibranchial placode is cut through its anterior end. The small mass of cells lying between placode, endoderm, and geniculate ganglion may belong either to the placode or to the ganglion since the ganglion has a rather indefinite outline.

Fig. 43 from an 8.3 mm. embryo, shows the maximum size of the placode at this age. The indefinite outline of the geniculate ganglion at this stage renders the line of separation between the placode and the geniculate ganglion difficult of determination.


40 ×350
Ec.
Pr.L.L.
E.P.VII
Gen.


41 ×350
Pr.L.L.
Gen.
Ec.
E.P.VII
En.


42 ×350
Gen.
E.P.VII
En.
Ec.

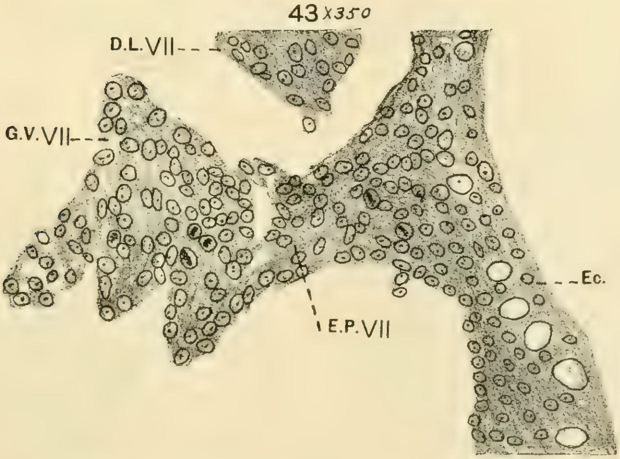

43 ×350
D.L.VII
G.V.VII
Ec.
E.P.VII

PLATE 9

Fig. 44 from an 8.8 mm. embryo shows the earliest trace of cells in the IX that would ordinarily be identified as placode. Succeeding series do not show it to be continuous and it differs from other placodes in having the ectodermic shelf extend mesially to the placodal cells.

Fig. 45 from a 9.7 mm. embryo shows, in the IX, the earliest trace of the placode that can be followed continuously in later series up to the time of its detachment.

Fig. 46 from a 10 mm. embryo shows the 'comma' stage of IX immediately after or during its detachment from the epidermis. The placode is not sharply differentiated from the general visceral, but includes at least all of the ventro-laterally projecting mass. The whole ganglion rests upon the ectodermic shelf.

Fig. 47 from a 12.4 mm. embryo passes through the anterior end of the placodal mass of cells of IX and is almost surrounded by the general visceral cells.

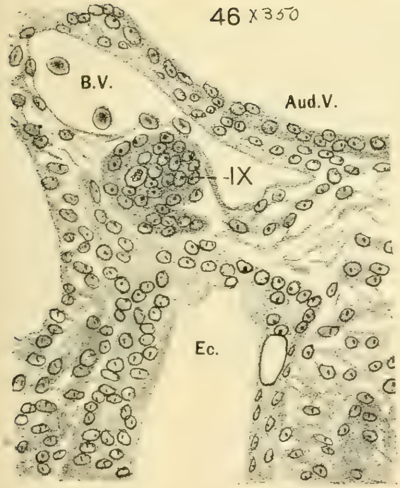
Fig. 48 from a 12.4 mm. embryo passes through the point where the lateral spur of placodal cells is present and the remainder of the placodal cells are only partially incorporated and occupy the ventral side of the ganglion. Fig. 48 lies three sections back of fig. 47.

Fig. 49 from a 10 mm. embryo shows the early stage of the epibranchial placode of the first branchial ganglion of X still attached to the ectoderm.

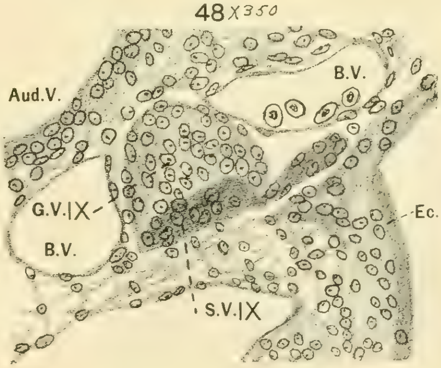
44 ×350

45 ×350

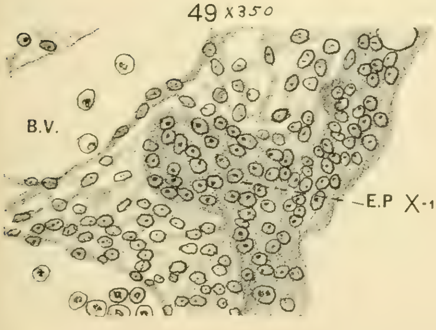
46 ×350



48 ×350



49 ×350



47 ×350

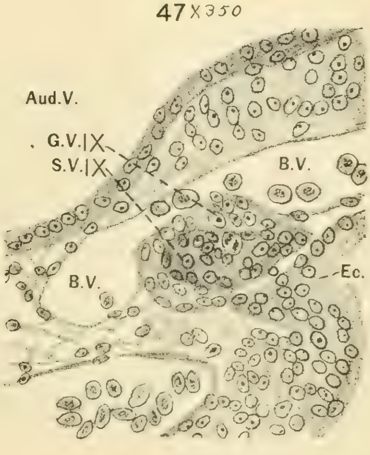


PLATE 10

Fig. 50 from a 11.5 mm. embryo is taken through the 'comma' stage of the placode of the first branchial ganglion of X and shows the relation assumed by the placodal and general visceral cells in a ganglion where they are approximately equal in number.

Fig. 51 from a 10 mm. embryo shows an early stage of the epibranchial placode of the second branchial ganglion of the X at a stage where it is still attached to the ectoderm.

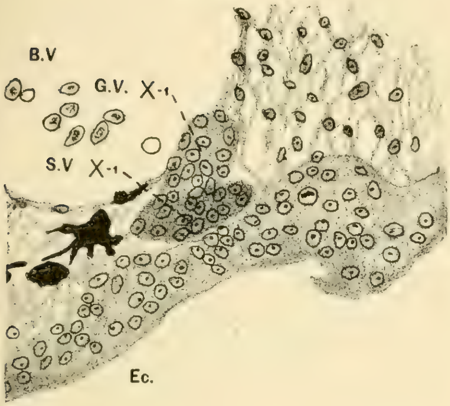
Fig. 52 from a 10.9 mm. embryo shows a late stage in the second epibranchial ganglion of X. The incorporation of placodal cells is not complete, there being a ventro-lateral spur of placodal cells and, contrary to the usual rule, the placodal cells lie chiefly on the lateral portion of the general visceral ganglion.

Fig. 53 from a 10.9 mm. embryo shows an early stage before detachment of the epibranchial placode of the third branchial ganglion.

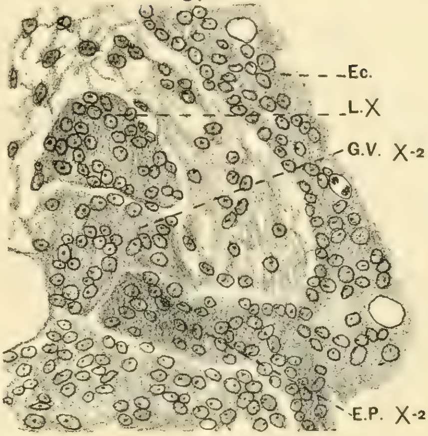
Fig. 54 from a 11.5 mm. shows the process of incorporation of the third epibranchial placode into the ganglion of the X. The lateral spur is still present.

Fig. 55 from a 12.9 mm. embryo shows an early stage in the formation of the fourth epibranchial placode of the X. The general visceral cells are so numerous that the relation seems to be one of contact only but later series show that there is an actual contribution by the placode of the ganglion.

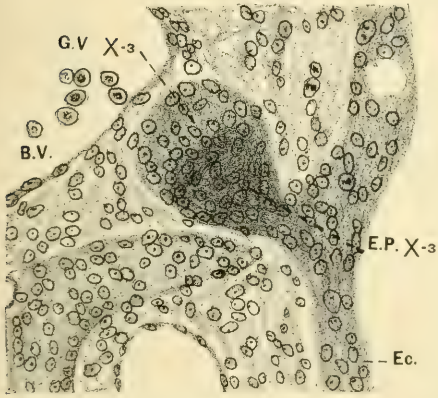
50 × 350



51 × 350



53 × 350



52 × 350

54 × 350

55 × 350

A COMPARISON OF THE EUROPEAN NORWAY AND ALBINO RATS (MUS NORVEGICUS AND MUS NORVEGICUS ALBINUS) WITH THOSE OF NORTH AMERICA IN RESPECT TO THE WEIGHT OF THE CENTRAL NERVOUS SYSTEM AND TO CRANIAL CAPACITY

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FIVE FIGURES

In a paper recently published (Donaldson and Hatai, '11) the fact that the domesticated albino rat (Mus norvegicus albinus) of our laboratory colony has a relatively smaller central nervous system than the wild Norway (Mus norvegicus) from which it is derived, has been presented and examined in some detail. This difference between the two forms has been known to us for several years and ever since it was first appreciated we have been in search of a satisfactory explanation for it.

In the paper just cited, the conclusion is reached that this difference represents one effect of domestication on the albino rat, but in order to justify this conclusion, it will be necessary not only to analyse domestication into its main factors, but also to test other explanatory suggestions which have been made.

One such suggestion is to the effect that the small relative weight of the central nervous system in the domesticated Albino is due to the fact that the Albino has been derived from a strain of the wild Norway in which the central nervous system was also relatively small. It was to test this suggestion that the present study has been made.

At the same time we recognize that this question represents one aspect only of the larger problem of the possible variations

which the Norway rat has undergone in its migration across Europe and over seas to the Americas.

From our experience with the wild Norways of North America, as found in Chicago and Philadelphia, there is no reason to think that such an established strain exists in the United States, although there appear to be slight differences characteristic for the rats from different stations, and in one instance the rats from a restricted locality near Philadelphia showed a brain weight decidedly lower than that of our standard series. We do not look on this latter group however as representing an established strain.

It was thought possible nevertheless that such a strain might be found in western Europe and I decided therefore in the summer of 1909 to test the matter by collecting both Norway and albino rats from Vienna, Paris and London in order to compare these in respect to their central nervous systems with specimens of both forms as observed in Philadelphia.

Historically, nothing is known of the albino variety of the Norway rat, not even whether the Albinos found in Europe and those in North America have had a common origin.

Concerning the wild Norway rat, we are a trifle better informed. Mus norvegicus is reported by Pallas to have entered Europe by way of southern Russia about the beginning of the eighteenth century. It arrived in England, by ships, about 1728–1729 and in Paris about 1758, but of its first arrival in Vienna, I have found no mention. The date of its arrival on the eastern seaboard of the United States was about 1775 (Lantz, '09). Thus the wild Norway rat has been in Europe for nearly two hundred years, and in the eastern United States for about a hundred and thirty-five years. It seems most probable that the albino variety has been established since the appearance of the wild Norway rat in Europe.

For the opportunity to make these studies on the rat at the several European stations, I was indebted to the courtesy of colleagues in each city, and it is a pleasure to present here my thanks to all of them for their unfailing kindness and interest.

At Vienna, Professor Obersteiner obtained for me a table in

the Physiological Institute directed by Professor v. Exner. Through Dr. Przibram, Director of the Biologische Versuchsanstalt, arrangements were made to get Norway rats and ultimately Dr. Przibram, beside presenting me with some of his own Albinos, kindly allowed his assistant, Dr. Megusar, to make for me the collection and first measurements of the animals needed to complete the Vienna series. My thanks are specially due to Dr. Megusar for the precision and care with which this work was done.

In Paris, Professor Lapicque obtained for me a table in the Physiological Laboratory at the Sorbonne, and in every way aided my plans.

Finally, in London, Sir Victor Horsley courteously allowed me to work in his own laboratory and through his assistants arranged for the supply of animals.

It may be noted in passing that rats in these several cities are most readily obtained through local dog fanciers who usually control a supply used for the higher education of their terriers.

Norway rats were hard to get in Vienna. Possibly the war of extermination waged against them about 1898, when public interest was aroused by a laboratory outbreak of plague, has served to check their increase.

In Paris the rats were easier to obtain, but bore evidence of having been caught in rather unsavory surroundings. In London they were very numerous. I was offered a thousand in three days, and moreover Mus rattus alexandrinus and Mus rattus, the old English black rat, were also to be had—both kinds in large numbers.

The general plan of this investigation was the following: To collect at each of the three foreign cities about one hundred Norway rats—and a smaller number of Albinos; to record the body weight and body length of each individual, and in a few cases to remove and weigh the central nervous system on the spot. In the majority of cases however, it was planned to preserve the heads only.

Later the skulls were to be prepared in this laboratory, the cranial capacity determined and the data from the several series

as thus obtained, used as a basis for determining the relative weight of the brain.

Moreover, corresponding series of animals from Philadelphia were to be prepared by a like technique and these data in turn used as standards with which to compare the European records. This plan was carried out, and the results furnish the material for the discussion which follows.

The constitution of the several series of specimens is shown in tables 1 to 11 inclusive. These tables give by series the number of individuals and the range in body weight for each sex separately.

TABLE 1

Mus norvegicus from Vienna

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	38	78–400
Females	55	68–354

TABLE 2

Mus norvegicus albinus from Vienna

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	4	180–292
Females	6	142–204

TABLE 3

Mus norvegicus from Paris

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	50	64–391
Females	46	86–389

TABLE 4

Mus norvegicus from Paris. Used for direct determination of the weight of the central nervous system

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	7	183–328
Females	2	154–234

TABLE 5

Mus norvegicus albinus from Paris

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	8	86–203
Females	2	94–109

TABLE 6

Mus norvegicus from London

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	51	72–385
Females	45	88–382

TABLE 7

Mus norvegicus from London. Used for the direct determination of the weight of the central nervous system

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	5	157–305
Females	4	173–215

TABLE 8

Mus norvegicus albinus from London

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	5	106–286
Females	5	150–250

TABLE 9

Mus norvegicus from Philadelphia

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	49	104–533
Females	46	75–457

TABLE 10

Mus norvegicus albinus from Philadelphia. Prepared in 1910-11

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	9	101–194
Females	11	72–124

TABLE 11

Mus norvegicus albinus from Philadelphia and Chicago. Material prepared in 1907 by Dr. Hatai

	NUMBER	RANGE IN BODY WEIGHT
		grams
Males	41	112–292
Females	35	108–253

An examination of the data for the four longer series—namely those for the wild Norways—used in the determination of cranial capacity (tables 1, 3, 6 and 9) suggests several comments. In collecting these series no selection by either size or sex was made by me, and yet the proportion of the sexes is rather similar in all four. In the three European series, the range in body weight is also similar, while distinctly heavier rats are found in the Philadelphia series. It may be remarked that in this latter case we received every animal caught, while it is just possible that in the case of some of the European series, the dealers reserved the very largest animals for their own use, and in this way modified the range in body weight. However that may be, the data as they stand show that the several series were taken from rat populations which were similar in their general composition.

TECHNIQUE

In all cases the rats were brought alive to the laboratory, killed with chloroform, weighed and the body length taken (see Donaldson, '09).

Then, either the central nervous system was removed and weighed, according to the usual technique (Donaldson and Hatai, '11) or the head cut off. This latter was marked with a numbered metal tag and preserved in 60 per cent alcohol, pending the preparation of the skull. As all the series were treated in the same manner, the effects of the 60 per cent alcohol in modifying the capacity of the cranium should be similar, provided of course that the composition of the skull bones was also the same.

The skulls were cleaned by immersion for from 5 to 6 hours at a temperature of 90° C. in 100 cc. of a 2 per cent watery solution of commercial 'gold dust washing powder.'[1] The very young skulls required less time and a half strength solution.

The softened tissues were removed with a bone scraper. The skull was then marked with water proof ink, dried in the air, and the foramina at the base plugged with a minimal quantity of universal cement to prevent the escape of the shot later used for determining the cranial capacity.

To determine the capacity of the dried cranium[2] it was filled with new number eleven shot. These shot are 0.06 of an inch in diameter and weigh individually from 0.0203 to 0.0215 grams and therefore run from 47 to 49 to the gram, with a mean of 48.

In filling the cranium the following method was used: The cranium was held vertically between the thumb and forefinger of the left hand with the ventral side towards the observer. The shot was poured from a small aluminum beaker into the cranium through the foramen magnum until it was nearly filled, then transferring the cranium to the right hand, and holding it vertically between the thumb and middle finger with the index finger closing

[1] 'Gold dust washing powder' consists of about 45 per cent sodium carbonate, 30 per cent soap powder and 25 per cent water.

[2] Departing from the strict anatomical nomenclature, and following the usage of the anthropologists, we shall here employ the term "cranium" for the skull without the mandible (see Cunningham, '09, p. 103, note 1).

the foramen magnum, the right hand was gently struck three times against the left. This was done to pack any shot which might have been caught on irregularities within the cranium.[3]

The cranium was then transferred to the left hand again and held as before while more shot were poured in. These were packed with a small spatula and finally pressed down with the forefinger of the right hand. The filling was such that the shot was slightly heaped in the center of the foramen so as to rise a little above the level of its edge. When thus filled, each cranium was placed vertically, nose down, in a small weighing bottle, so that no shot should be lost through accidental jarring.

The filled cranium was next weighed to the tenth of a milligram and the weight of the shot computed by subtracting from the weight of the filled cranium the weight of the empty cranium as previously determined. The cranium was then emptied and again filled and weighed, and if the two weights of the shot were within one per cent of one another, they were deemed satisfactory. In 84 per cent of the cases two weighings only were necessary. When the discrepancy was greater than 1 per cent in the first instance, and more than two weighings were required, the two determinations in closest agreement were selected for averaging, and the number thus obtained was that recorded. In this connection it may be noted that in the nine series of crania measured in the course of this investigation, the mean difference between the two weighings of shot which were used as the basis for the averages, was 0.6 per cent. This corresponds to a mean difference of from three to five shot according to the total weight of the shot required to fill the cranium.

[3] As serving to illustrate the pitfalls besetting the determination of cranial capacity by the use of shot, I may call attention to the fact that when a container has been filled, it does not always follow that any disturbance of the contained shot will decrease its volume. For example: if a glass measuring cylinder graduated to hold 10 cc. and having an internal diameter of 1 cm. is exactly filled to the upper limit of the graduation with number 11 shot, poured into it in a steady stream, so that the filling requires about fifteen seconds, and then the mouth of the cylinder is closed with the thumb and the cylinder once inverted, the shot having a run within the cylinder of about 5 cm., it is found that the volume of the shot is *increased* by this treatment about 0.5 cc. or 5 per cent. If the cylinder be now gently tapped on the bottom eight or ten times, the volume of the shot again diminishes to its original value.

This technique is essentially that which was used by Hatai ('07) but has been given here in detail because in the determination of cranial capacities, concordant results can be obtained only by the strictest adherence to a uniform procedure.

The various measurements made by the foregoing methods have been recorded and tabulated in detail and the individual records are on file at the Wistar Institute where they are at the service of other investigators. In the presentation which follows however, we shall use only mean values, grouping the records for each series by mean body weights within weight groups differing by 50 grams. The smallest weight group includes the individuals between 51 to 100 grams in body weight, and the largest those between 351 to 400 grams. The higher body weights appearing in the Philadelphia Norway series have not been considered because there are no European records with which to compare them.

BODY FORM

Before taking up the records touching the central nervous system, it is important to know whether the several series to be examined are composed of animals having a like body form. The most general test for likeness in form is the relation of body length to body weight. The data for such a test are contained in tables 12 and 13. For our present purpose therefore we select from these tables the records giving the mean body weights and mean body lengths for each weight group of each series.

In chart 1 these data for body length both for the Norways and Albinos are entered in relation to the standard curves for body length based on Philadelphia material (Donaldson '09 and Donaldson and Hatai, '11). A study of chart 1 shows that the European series of both forms agree fairly with the standard records as represented by the continuous curves in the chart.

The records for the European Norways run somewhat below the standard. This is especially noticeable in the case of the Vienna and Paris series. The records for the European Albinos agree very closely with the standard curve based on the Philadelphia Albinos. On the whole, the accordance between the

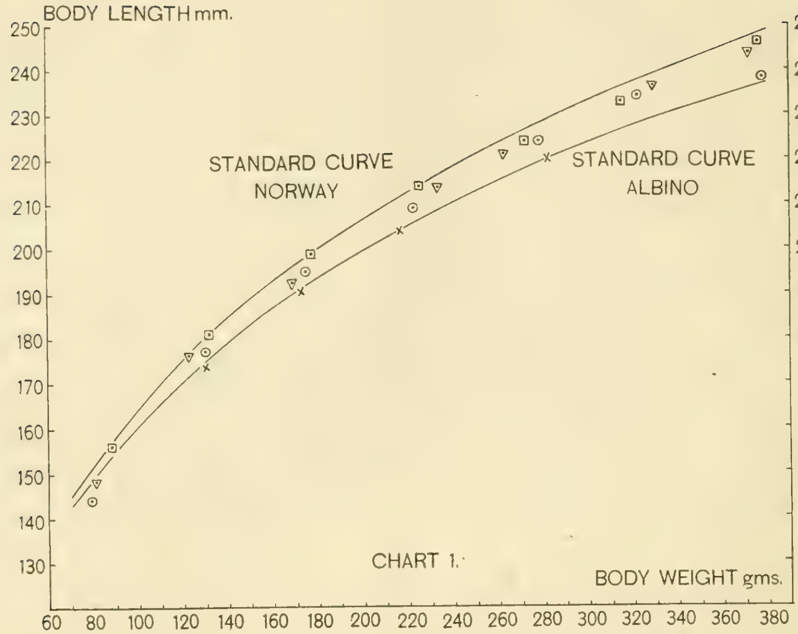

Chart 1, giving the body length in millimeters for each of the three European Norway series separately in relation to the standard curve for the body length of the Philadelphia Norways. Also giving the body length for the three European albino series combined, in relation to the standard curve for the body length of the Philadelphia Albinos. Norways: ⊙ = Paris series, ⊡ = London series, ▽ = Vienna series. Albinos: × = European series (combined).

European series and the standards is close. Hence we conclude that in body form, the European Norways are similar to one another and to the Philadelphia standard, and that the same relations are true for the European and Philadelphia Albinos.

Since the completion of this manuscript, a paper by Chisolm ('11) has appeared in which he compares the body length of tame albino and piebald rats of London with those of the Philadelphia Albinos as given by me in an earlier paper (Donaldson, '09).

Chisolm's measurements agree with mine very well up to about 100 grams of body weight and from there on fall steadily below the Philadelphia records with a maximum deficiency of about 5 per cent. It seems probable however that this difference is mainly due to the difference in the method of taking the body length.

TABLE 12

Giving for each body weight group of Mus norvegicus the mean values for all the individuals from each of the four stations. Below each weight group is also given the mean for the European series alone.

Records from Philadelphia = A; Paris = P; London = L; Vienna = V

WEIGHT GROUP	SERIES	NUMBER OF CASES BY SEX		MEAN BODY WEIGHT	MEAN BODY LENGTH	MEAN CRANIAL CAPACITY
		M.	F.			
				grams	*mm.*	*cc.*
51–100	A	0	1	74.9	157	1.575
	P	1	2	79.3	142	1.533
	L	3	4	87.8	156	1.509
	V	3	4	80.6	148	1.567
Mean of European series	..	..	......	**82.5**	**149**	**1.536**
101–150	A	2	3	128.2	179	1.828
	P	10	7	130.1	177	1.795
	L	5	7	126.1	179	1.653
	V	4	5	126.0	178	1.694
Mean of European series	......	......	......	**127.4**	**178**	**1.714**
151–200	A	5	11	179.5	194	1.897
	P	3	10	173.8	195	1.894
	L	8	7	177.1	199	1.898
	V	8	20	171.4	193	1.788
Mean of European series	......	......	......	**174.1**	**196**	**1.860**
201–250	A	11	6	226.2	214	2.000
	P	11	9	221.5	209	2.002
	L	14	8	224.7	214	1.980
	V	9	14	223.9	211	1.936
Mean of European series	......	......	......	**223.4**	**211**	**1.972**
251–300	A	5	8	282.9	225	2.131
	P	15	10	277.8	224	2.129
	L	12	13	272.3	224	2.053
	V	8	7	261.7	221	1.978
Mean of European series	......	......	......	**270.6**	**223**	**2.053**
301–350	A	4	13	326.2	237	2.210
	P	7	5	322.2	234	2.205
	L	8	5	320.7	234	2.090
	V	2	4	322.7	235	2.154
Mean of European series	......	......	......	**321.9**	**234**	**2.149**
351–400	A	13	2	377.7	246	2.285
	P	3	3	377.6	238	2.248
	L	3	1	375.3	246	2.318
	V	4	1	377.9	245	2.225
Mean of European series	......	......	......	**377.0**	**243**	**2.264**

TABLE 13

Giving for each body weight group of Mus norvegicus albinus the mean values for all the individuals from each of the four stations. Below each weight group is also given the mean for the European series, so far as represented in the group.

Records from Philadelphia = A; Paris = P; London = L; Vienna = V

WEIGHT GROUP	SERIES	NUMBER OF CASES BY SEX		MEAN BODY WEIGHT	MEAN BODY LENGTH	MEAN CRANIAL CAPACITY
		M.	F.			
				grams	*mm.*	*cc.*
51–100	A	0	5	87.8	157	1.364
	P	1	1	90.0	158	1.443
	L					
	V					
Paris series				**90.0**	**158**	**1.443**
101–150	A	11	15	127.3	173	1.524
	P	5	1	117.6	175	1.560
	L	1	1	128.0	171	1.494
	V	0	2	145.5	174	1.497
Mean of European series				**130.4**	**174**	**1.517**
151–200	A	19	23	174.2	193	1.661
	P	1	0	173.0	194	1.671
	L	0	2	184.0	196	1.646
	V	1	3	160.2	184	1.638
Mean of European series				**172.4**	**191**	**1.652**
201–250	A	13	2	218.6		1.743
	P	0	1	203.0	203	1.822
	L	1	1	234.7	209	1.758
	V	1	1	213.5	199	1.704
Mean of European series				**217.1**	**204**	**1.761**
251–300	A	7	1	275.9		1.768
	P					
	L	3	1	276.6	218	1.805
	V	2	0	289.4	222	1.916
Mean of London and Vienna series				**283.0**	**220**	**1.860**

Chisolm used the posterior margin of the symphysis pubis, while I used the anus, as the caudal limit of the measurement and thus my measurements must give slightly greater values than were found by him. When correction is made for this difference in method, the two series of records agree nicely and give a welcome

confirmation of the statements just made concerning the similarity of the albinos on the two sides of the Atlantic.

In this case of body length, as in that of the other data on the weight of the brain and spinal cord and on cranial capacity, to be presented later, the records are for the *two sexes combined,* since the differences according to sex are so small as to be negligible for the present investigation (Donaldson and Hatai, '11).

WEIGHT OF THE CENTRAL NERVOUS SYSTEM

The establishment among the several series of the similarity in body form clears the way for the study of the weight of the central nervous system in these same series. The method of direct weighing is the simplest and, as it has turned out, the most satisfactory method of attacking the problem before us. Had I to repeat this investigation I should make only direct determinations on the weight of the brain and spinal cord and omit those on cranial capacity, but when the investigation was begun, it was thought that the longer series of cranial capacity determinations would be more valuable than a shorter series of direct weighings, which require withal more time to make; and my time was limited. Nevertheless I had planned from the first to make direct weighings of the central nervous system for very short series of the Norways at each of the European stations. This was not done at Vienna because specimens for the purpose could not be obtained during the period of my stay there, but it was done for both the Paris and the London Norways. It is these latter records which are now to be considered. The data are given in tables 14 and 15.

When these data for the weight of the brain and the weight of the spinal cord are entered in relation to the corresponding standard curves for brain weight based on body weight (Donaldson and Hatai, '11) as given in chart 2, it is plain that both the European series closely agree with the respective standards for brain and spinal cord. The mean percentage deviation of the values as observed, from those of the standards, are given in table 16, A.

For comparison and control we have also given in table 16, B, the mean percentage deviation of the values as observed from

values for the brain weight when based on the body length (Donaldson and Hatai, '11, tables 5 and 8). It will be noted that the two series of results agree very well.

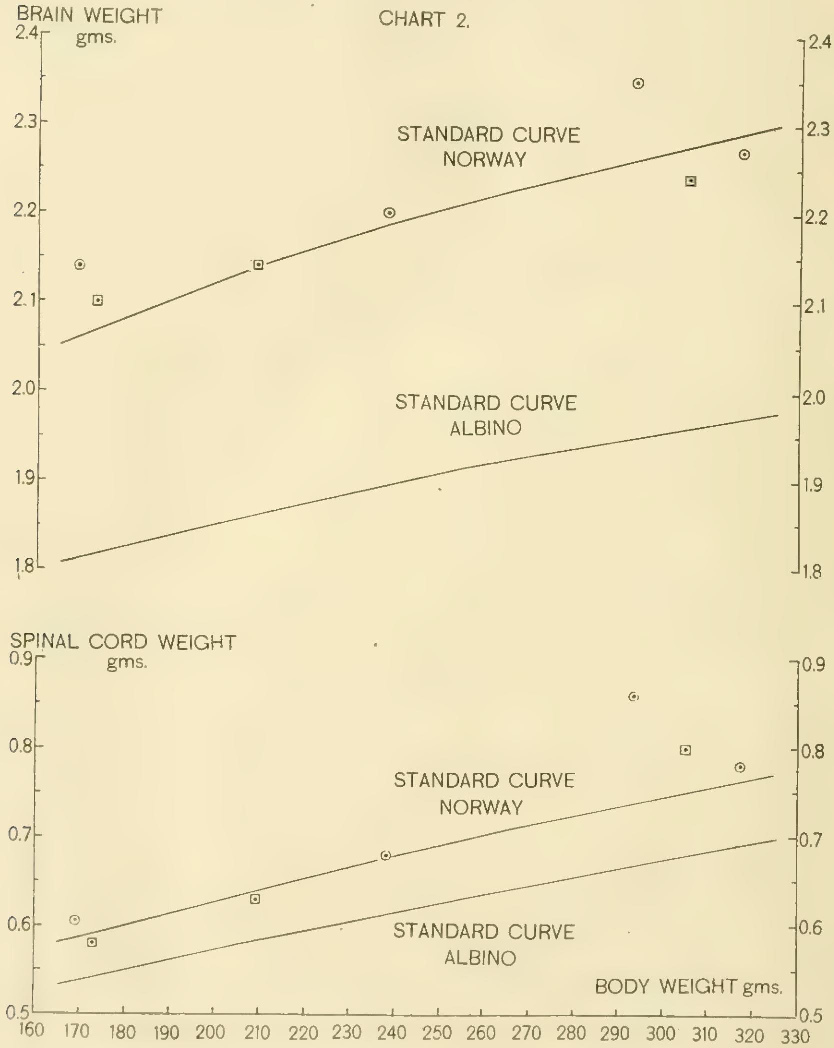

Chart 2, brain and cord weight of Paris and of London Mus norvegicus, entered in relation to the respective standard curves for the Philadelphia data. For further control, the corresponding standard curves for the Philadelphia Albinos are also given on the chart. ○ = Paris series. □ = London series.

TABLE 14

Mus norvegicus, Paris. Weight of brain and spinal cord

WEIGHT GROUP	NUMBER OF CASES BY SEX		BODY WEIGHT	BODY LENGTH	WEIGHT OF	
	M.	F.			Brain	Spinal cord
			grams	*mm.*	*grams*	*grams*
151–200	1	1	168.6	191	2.1410	0.6045
201–250	3	1	237.7	219	2.2028	0.6838
251–300	1	0	293.0	230	2.3514	0.8630*
301–350	2	0	317.0	227	2.2733	0.7785

TABLE 15

Mus norvegicus, London. Weight of brain and spinal cord

WEIGHT GROUP	NUMBER OF CASES BY SEX		BODY WEIGHT	BODY LENGTH	WEIGHT OF	
	M.	F.			Brain	Spinal cord
			grams	*mm.*	*grams*	*grams*
151–200	3	1	173.0	197	2.0959	0.5815
201–250	1	3	208.5	210	2.1404	0.6328
251–300						
301–350	1	0	305.0	237	2.2440	0.8031

*As this record for the spinal cord, which is correct, is also exceptional and is based on a single case only, the record is not used in computing the percentage deviations given in table 16.

TABLE 16

Mus norvegicus. Mean percentage deviation of the weight of brain and spinal cord in the Paris and London series from the standard values for the Philadelphia Norways

A. Comparison on the basis of body weight, as given in table 2

SERIES	MEAN PERCENTAGE DEVIATION	
	Brain	Spinal cord
Paris	+2.0	+2.7
London	0.0	+1.4
Average	+1.0	+2.0
B. Comparison on the basis of body length		
Paris	+2.9	+4.5
London	−0.2	+0.9
Average	+1.4	+2.7
Average of both A and B	+1.2	+2.4

The average of the deviations as given in tables 16, A and 16, B, is the figure used in the subsequent discussion.

According to these determinations, it is plain that both the Paris and London Norways have central nervous systems (brain and spinal cord) slightly heavier than that of the standard Philadelphia Norways. The deviations of the European forms from the standard are so small however, even in the case of the Paris series, that they are probably not significant. It is possible to conclude therefore that the Paris and London rats do not have central nervous systems of *less* relative weight than those found in the Philadelphia Norways, while as the records stand, the relative weight is really slightly higher. Although, as has been explained, the corresponding data for the Vienna Norways are lacking, we shall see further on that the true cranial capacity of the Vienna Norways is compatible with a brain weight about equal to that of the Philadelphia series or a trifle below it—but probably not deviating to any significant degree.

Although the data for the percentage of water in the brain and spinal cord are not presented here because the determinations could not be made abroad with all the desired precautions, yet the data as they stand agree well with those for the American Norway as previously determined (Donaldson and Hatai, '11). In this respect then the European and American Norways are again in agreement.

The foregoing determinations are highly important for the control and interpretation of the data on cranial capacity since these latter, as we shall see, cannot be taken at their face value, but must be corrected for shrinkage, which varies not only according to age, but also according to other less evident conditions. The characters of these crania, so far as they are indicated by weight, have been discussed elsewhere (Donaldson '12).

CRANIAL CAPACITY

In determining the cranial capacity by the methods given earlier we obtain in the first instance the weight of the shot necessary to fill the cranium. It was found that one cubic centimeter of the

shot used weighed 6.354 grams ± 0.020, hence the observed weight in grams is transformed into volumes in cubic centimeters by dividing the weight of the shot by 6.354. In tables 12 and 13 the data on the cranial capacity in cubic centimeters are presented for each one of the entries.

Taking the entries in table 12, which refer to the wild Norways, we wish in the first instance to learn how the cranial capacities of the several European series are related to that of the Phila-

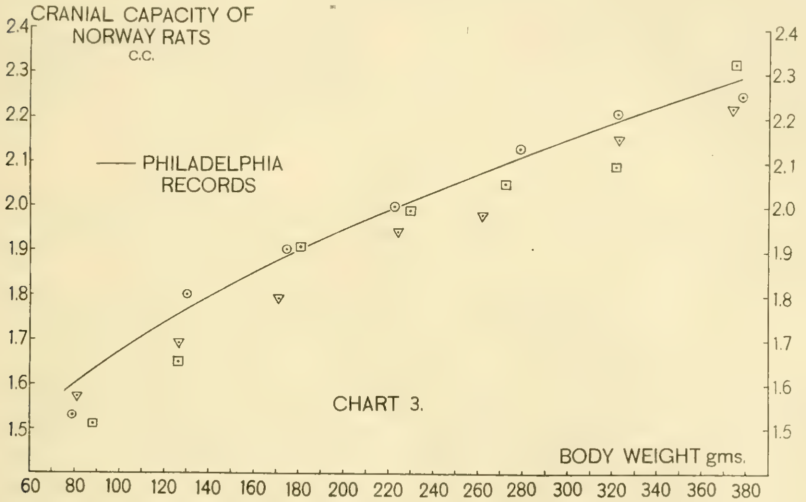

Chart 3, showing the cranial capacity in cubic centimeters for the four Norway series. The values for the Philadelphia series are represented by a curve computed from formula (1). The values for the European series are entered separately. ○ = Paris series, □ = London series, ▽ = Vienna series.

delphia series taken as a standard. To show this, the European data have been plotted by groups on chart 3, while the Philadelphia records are represented by a continuous line showing the computed values. For the formula for this line, I am indebted to Dr. Hatai.

The relation between the computed and the observed values in the Philadelphia series is close. Except for the second entry at 128.2 grams, which entry is a trifle high, the observed data for the cranial capacity of the Philadelphia Norways show an orderly

increase. Assuming then that the terminal values are correct, that the second entry is aberrant and that the intermediate values are approximately normal, Dr. Hatai determined the formula for the curve which fits the observations. This formula is as follows:

$$y = 0.00105\,x + 0.548 \log x + 0.476 \ldots\ldots\ldots\ldots (1)$$

where x equals the body weight and y the cranial capacity in cubic centimeters.

Examination of chart 3 shows at once that the three series of the European records do not run exactly with the line representing the Philadelphia series. Inspection shows that the order of the

TABLE 17

Mus norvegicus. Showing the mean percentage deviation in cranial capacity for each of the three European Norway series from the Philadelphia series taken as a standard

SERIES	MEAN PERCENTAGE DEVIATION
Paris	−0.4
London	−2.5
Vienna	−3.3
Average	−2.1

mean values from highest to lowest is Philadelphia, Paris, London and Vienna. If then we compute the mean deviation from the Philadelphia records for each of the European series given on the chart, we obtain the values entered in table 17.

As table 17 shows, the average of the mean deviations for the cranial capacity of the European Norway series combined is −2.1 per cent. It thus appears that all the European series for the Norway rat exhibit a smaller cranial capacity than the Philadelphia series and that the most marked deviation occurs in the case of the London and Vienna series where the deficiency is 2.5 and 3.3 per cent respectively.

For the Albinos, the Philadelphia series are also taken as the standard. The curve is represented by the continuous line in

chart 4. This is calculated from formula (2). The observed value for the smallest weight group of the Philadelphia series was considered normal (see table 13) but that for the largest weight group was taken midway between the value for the Vienna series and that observed for the Philadelphia series. The intermediate determination, at a body weight of 175 grams, was also taken midway between the observed Philadelphia record and the corresponding record for the London series—which is here low.

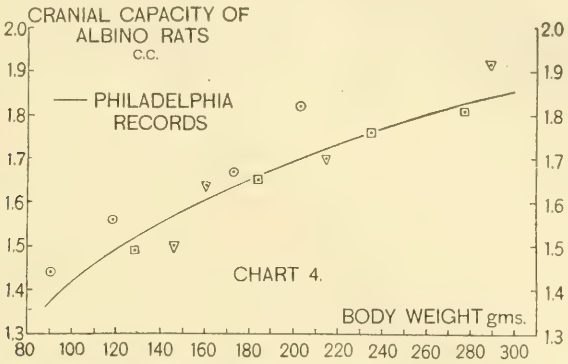

Chart 4, showing the cranial capacity in cubic centimeters for the four albino series. The values for the Philadelphia series are represented by a curve computed from formula (2). The values for the European series are entered separately. ○ = Paris series, □ = London series, ▽ = Vienna series.

Using these three points, Dr Hatai has kindly devised the following formula:

$$y = 1.02 \log x - 0.00027\, x - 0.596 \ldots\ldots\ldots\ldots (2)$$

where x is the body weight and y the cranial capacity.

The curve thus obtained is the one entered in chart 4 and used as the standard from which to compute the deviations of the records for the European series. The European albino records follow in about the same order as the records for the Norways, i.e., the Paris series give the highest mean value, being above the standard, while the London and Vienna series are slightly below it.

These relations suggest that the conditions in the three European stations which act to modify ultimately the cranial capaci-

ty of the dried crania also act in a like manner on both the wild Norways and the domesticated Albinos. Calculation shows that the combined records for the European Albinos run somewhat *above* those for the Philadelphia Albinos. The figures are given in table 18.

These results suggest for consideration several important questions. As is seen in table 17, the mean cranial capacity of the Paris Norway series is 0.4 per cent less than that of the Philadelphia series, while from table 16, we see that the Paris brain weight is about 2.5 per cent greater. It would appear from this that the Paris crania had shrunk about 3 per cent more than the Philadelphia crania. Again table 17 shows the capacity of the

TABLE 18

Mus norvegicus albinus. Showing the mean percentage deviation in cranial capacity of each of the three European albino series from the Philadelphia series taken as a standard

SERIES	MEAN PERCENTAGE DEVIATION
Paris	+4.9
London	−0.1
Vienna	−0.1
Average	+1.6

London crania to be about 2.5 per cent less than that of the Philadelphia crania while the brain weights are nearly identical; once more a greater relative shrinkage in the London series. The same thing has probably happened in the case of the Vienna series, but for this series the brain weight determinations are lacking.

The albino records must also represent crania which have suffered more or less shrinkage characteristic for the series to which they belong, but as table 18 shows, two of the series run close to the Philadelphia standard, though slightly below it, while one, the Paris series, is clearly above it. As the preparatory treatment of all the skulls was the same, these variations in the amount of shrinkage must find their explanations in variations in the composition of the skull bones—as represented by the amount of

water, organic matter and salts in them and by their thickness. Watson ('06) has shown that the constitution of the osseous system of the rat can be significantly modified by diet.

In the meantime it is clear that we cannot infer that the brain weights in the several Norway series are related precisely as are the cranial capacities.

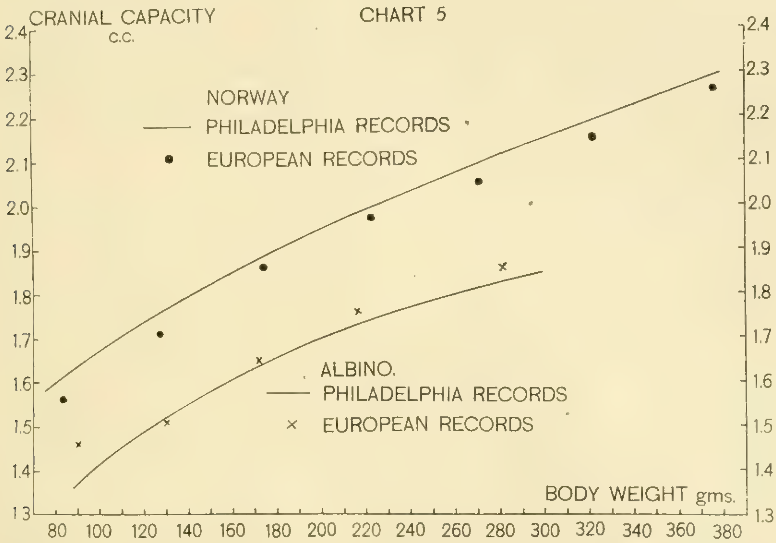

Chart 5, cranial capacity based on European series only. Showing the cranial capacity in cubic centimeters of the European series of Norways all combined, contrasted with the computed value (formula 1) for the Philadelphia records and similarly of the European series of Albinos all combined, contrasted with the computed value (formula 2) for the Philadelphia records. ● = European series—Norway. × = European series—Albinos.

However, bearing in mind that the cranial capacities of the three European Norway series combined are probably as much as 2.1 per cent too low, and that so far as the tables 13 and 18 and chart 4 show, the capacities of the combined European albino series are slightly (1.6 per cent) above those for the Philadelphia Albinos, it is possible to make a general comparison between the two forms as found in Europe, in respect to their cranial capacity.

The data for this comparison are found in tables 12 and 13 and the averages for the European series there given are plotted

in chart 5. In making these averages, the mean values for each series are taken as they stand in the tables and are not weighted for the number of cases to which each applies.

When the records for the European albino cranial capacity are thus compared with those for the European norway, it is evident that there are considerable differences between the two. The approximate values of these differences can be determined in the following way:

If we take as the standard the body weight values of the European albinos as given in each body weight group in table 13 and

TABLE 19

Showing the percentage values of the differences in cranial capacity between the European Albinos and the European Norways of like body weights. See chart 5. The values in the fourth column have been read from chart 5

WEIGHT GROUP	MEAN BODY WEIGHT	MEAN CRANIAL CAPACITY		PERCENTAGE DIFFERENCE IN FAVOR OF THE NORWAYS
		Albinos	Norways	
	grams			
51–100	90.0	1.443*	1.570	(8.8)
101–150	130.4	1.517	1.725	13.7
151–200	172.4	1.652	1.860	12.6
201–250	217.1	1.761	1.960	11.1
251–300	283.0	1.860	2.070	11.3
Average				12.2

* There is in this weight group only the Paris record, see table 13 and chart 4. The Paris record, which runs high, raises this value and hence diminishes the percentage difference given in the last column, thus slightly diminishing the general average. Hence the record for the 51–100 grams body weight group is omitted in making the final average.

enter also for these the cranial capacity as observed, and then read from chart 5 the corresponding observed values for the cranial capacity of the European Norways, and enter these readings also in table 19, we have before us the numbers which make it possible to compute the percentage differences between the albino and the Norway records (see table 19).

Table 19 shows the average difference between the combined European Albino and the combined European Norway records to be 12.2 per cent.

From the foregoing data on cranial capacity, we therefore conclude that there is no evidence that any of the European Norway series represent a small brained strain which might have been the source of the Albino variety.

The studies on the cranial capacity lead then to the same conclusion as was reached from the direct determinations of the weight of the central nervous system and thus the particular suggestion which we proposed to test is shown to be without support. There remain however some minor aspects of these results on cranial capacity which require further consideration.

TABLE 20

Showing the percentage values of the differences in brain weight between the Philadelphia Albinos and the Philadelphia Norways of like body weight—using the body weights for the European Albinos as given in table 19. Data in column 3 are calculated from the formula for the albino brain weight based on body weight (Donaldson '08). Data in column 4 are calculated from the formula for the Norway brain weight based on body weight (Donaldson and Hatai, '11)

WEIGHT GROUP	MEAN BODY WEIGHT	MEAN BRAIN WEIGHT		PERCENTAGE DIFFERENCE IN FAVOR OF THE NORWAYS
		Albinos	Norways	
51–100	90.0	1.640	1.827	(11.4)*
101–150	130.4	1.740	1.965	12.9
151–200	172.4	1.814	2.068	14.0
201–250	217.1	1.873	2.152	14.9
251–300	283.0	1.941	2.249	15.8
Average				14.4

* This entry omitted in making the final average so that this average may be directly compared with the final average in table 19.

Before it was recognized that the amount of shrinkage of the skulls in the different series was dissimilar, I had expected to find the percentage differences between the cranial capacities of the European Norways and European Albinos approximately equal to the percentage differences between the brain weights of the Philadelphia Norways and Philadelphia Albinos.

The data on brain weight which should be used for such a comparison are given in table 20.

This table was formed by using the same body weights as are given in table 19, but in place of the records for cranial capacity, entering those for brain weight. The average percentage difference in the brain weight is seen to be 14.4 per cent or 2.2 points higher than the corresponding value for the cranial capacity—which is 12.2 per cent.

We have already found that according to the data in table 17, the crania of the European Norways have shrunk 2.1 per cent more than those of the Philadelphia Norways, and those of the European Albinos 1.6 per cent less than those of the Philadelphia Albinos. Therefore if the average observed difference in the cranial capacity (12.2 per cent) were corrected for the excessive shrinkage of the European Norway crania, and the deficient shrinkage of the European Albino crania (i.e., 2.1 + 1.6 = + 3.7 per cent) it would give a value of (12.2 + 3.7) 15.9 per cent as that for the anticipated average difference in brain weight.

From table 20 the observed difference is seen to be 14.4 per cent. It is to be remembered however that this last is based on the brain weights of the Philadelphia forms and that we have already found, table 16, the brain weight of the European Norways (Paris and London series) to be 1.2 per cent greater than that of the Philadelphia Norways. If we use this as a correction, then the average difference in brain weights becomes (14.4 + 1.2 per cent) 15.6 per cent, or very close to the difference found (15.9 per cent) when the cranial capacities are corrected for the varying amounts of shrinkage shown by the several series.

Neither the nature of the data nor the method of comparison will justify us in pushing this argument in detail, but the general relations thus determined, clearly support the view that the difference in cranial capacity here found—when corrected for the unequal shrinkage of the crania in the two forms of the European rats, and for the slight excess in the brain weight of the European Norways, approximates the difference in brain weights which we should expect to find between the two European forms if the European and American rats were nearly alike in the relative weight of their central nervous systems.

There is still to be noted another dissimilarity between the percentage differences of the cranial capacities (table 19) as compared with the corresponding differences in brain weight (table 20). This dissimilarity also is due to the manner in which the cranium shrinks—in this instance according to age. It will be seen by looking at table 20 that the percentage difference in the brain weight increases regularly with increasing body weight. On the other hand, the corresponding records for cranial capacity in table 19 indicate a decrease with increasing body weight. Here again we should have expected the cranial capacity records to behave in the same way as the brain weight records, but they do not. We find the explanation for this disagreement in the shrinkage of the crania as influenced by age. The argument is as follows:

As is well known, in any series of crania those from the younger animals contain a larger proportion of water as well as more organic matter and have thinner bones and hence shrink relatively more when dried than do the crania from the older animals. We assume then that in any series of crania, loss of water and thickening of the bones increases with advancing age, and concomitantly, shrinkage on drying decreases with advancing age.

In the heaviest body weight group of the albinos (table 19) the crania are the more mature and so shrink less than the crania of the Norways of like body weight—which are somewhat less mature. This statement is based on the fact that the Norway rat, although it has probably the same span of life as the Albino (Donaldson and Hatai, '11) has nevertheless a much greater range in body weight and hence in general for a given body weight it must be younger than the corresponding Albinos, although the difference in the relative shrinkage for the heaviest body weight groups may be absolutely small. It follows from what has been said about the range of body weight in relation to the span of life in the Norways that an equal diminution in mean body weight, say 50 grams, implies a greater diminution in age for the Albino than for the Norway. According to the foregoing reasoning, this should be followed by a relatively increasing shrinkage in the albino crania, and this is the interpretation of the values given in the last column of table 19.

To bring together the observations and comments on cranial capacity which have just been presented, it appears that while the European Norways are shown to be distinct from the European Albinos as regards their cranial capacity, yet the interpretation of the direct observations is necessarily so modified by the shrinkage of the crania that in the case of any particular series, we can infer only in a general way from the cranial capacity to the brain weight.

CONCLUSIONS

1. In the case of the wild Norway rat, which entered western Europe about the beginning of the eighteenth century, and the eastern United States about 1775, the observations on the constitution of the populations, the general body form, the weight of the central nervous system and the cranial capacity, show that it is at the present time essentially similar in these respects in the two continents.

2. The observations on the albino rat from different stations indicate that this variety is also essentially similar in the two continents. Therefore there is no evidence to support the view that the Albino was derived from a strain of the Norway having a relatively small central nervous system.

3. Logically this result does not preclude the possibility of such an origin, but it does indicate on the other hand the absence of present evidence in favor of it.

4. That the Norway rats from the three stations in Europe are very similar to the Philadelphia Norways, despite the difference in station and the wandering of those that have crossed the sea, is probably due to the fact that the series of rats which have here been compared represent those which kept close to man, and all of which lived among food conditions and other surroundings characteristic of large cities; conditions and surroundings which are much the same in western Europe and eastern America.

A like explanation applies also to the similarity found among the Albinos from these two regions, and perhaps even more forcibly, as the treatment of these caged animals probably represents a still more uniform environment than that which the Norways experience in their wild life.

At some future time it will be of interest to examine the Norways of still other countries—those from India for example—where we should expect the food conditions to be quite different from those obtaining in western Europe and North America.

5. The capacity of the dried cranium of the rat is modified by age and by the amount of calcification to such an extent that the data for capacity cannot be transformed into those for brain weight without making correction for several fluctuating conditions.

6. The constancy of the foregoing characters in the rats (both Norway and Albino) from western Europe and easter America indicates that for the purpose of further general studies, the two populations may be considered as essentially similar.

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EXPERIMENTAL STUDIES OF PARALYSES IN DOGS AFTER MECHANICAL LESIONS IN THEIR SPINAL CORDS WITH A NOTE ON 'FUSION' ATTEMPTED IN THE CAUDA EQUINAS OR THE SCIATIC NERVES

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TWENTY-SEVEN FIGURES

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INTRODUCTION

Reasons for the research

The present series of experiments was begun in the spring of 1908 with the production of mechanical lesions in the lumbar spinal cords of dogs. The object was to bring about distinct motor paralyses. After the animals had reached permanent stages in the paralysis, so that there was no question about degeneration in the roots and peripheral nerves, it was planned to attempt to distort the nerve-patterns in these structures, using for this purpose a method heretofore apparently untried. This method is referred to as nerve 'fusion,' and consists simply in uniting two or more nerves by tying them together with absorbable ligatures.

In carrying out the plan given, it early became apparent that the subject of nerve 'fusion' was a research in itself, requiring many special experiments. Fusions were therefore attempted on normal nerves where no central lesions had been produced. A preliminary report of this work has already appeared[1], and we have been able to offer some evidence that after nerves are united by this method, a certain amount of distortion of pattern may take place.

However, even if changes in nerve-pattern are obtainable by the union of nerves which are normal, it does not follow that the same changes may be expected where there has been a central lesion. Fatty degeneration takes place in the paralyzed muscles and in the nerve trunks themselves, the degenerated tracts close up and become replaced by new connective tissue.

Therefore, the original design of producing central lesions and later trying nerve fusions has been carried out. In the course of this research two other issues have developed which proved to be of equal if not greater interest. It was shown that after a lesion was produced in the spinal cord by the method which will be outlined, a very characteristic spontaneous recovery took place similar to the early recovery often seen in infantile

[1] Feiss: Boston Medical and Surgical Journ., May 11, 1911.

paralysis, and it was found that the material furnished data for adding to the precision of our knowledge on the localization in the lumbo-sacral cord certain nuclei of the pelvic viscera and of the muscles of the hind limb and tail.

This report may therefore, be divided into three parts; in the first part attempting to account for the clinical improvement occurring after the lesion, in the second part showing the relation of the autopsy findings to the peripheral and visceral palsies produced, and in the third part discussing the subject of fusion and giving a brief account of some experiments in which, some months after the lesion, this procedure was attempted.

The production of the lesion

The lesions were made subdurally through a single trephine opening, usually at the 4th or 5th lumbar spine. The instrument for the purpose (fig. 1) was L-shaped, the longer arm forming the handle and the shorter shaped into a thin blade. This was entered through the dura in the median line and then moved laterally with sufficient force to crush the cord substance. Both unilateral and bilateral lesions were attempted. The operations were done under complete ether anesthesia with the usual precautions for asepsis. All told, lesions were attempted in some seventy-five animals, but of course, on account of various causes, including an epidemic of distemper, the majority of animals were lost.

Method of studying the surviving animals

Observations were made from day to day and notes were made on the changes. At intervals of 2 or 3 months, each dog was studied more systematically, according to the following routine:

1. Attitude
2. Gait. Nature of limp if present
3. Active movements: rump, hips, knees, ankles, paws and tail
4. Passive movements: rump, hips, knees, ankles and paws
5. Response to stimulation; sharp point applied to skin of various parts of limb
6. Response of paw to heat with immersion in hot water (heat-pain sense)
7. Temperature of skin between toes

8. Measurements. Circumferences above paw, ankle and knee
9. Reflexes: knee jerks; anal reflex (observed by inserting glass rod into anus)
10. Control of sphincters (based on ordinary clinical observations)
11. Response to faradism; applied to various parts of limb
12. Remarks
13. Photograph

In a few of the dogs data bearing on spinal localization could be obtained by direct stimulation of peripheral nerves, which was done at the time of the secondary operations of nerve fusion.

Study of autopsy material

The spinal cords were studied in twenty of the cases. In six of them proper identification of the roots could not be made, owing to scar. The other fourteen were each mounted on cardboard, and the roots spread out, stitched down, and properly numbered. The count was made from the thirteenth, using the last rib as a land-mark. Each mounted specimen was sketched and placed in Müller's fluid.

The boundaries of the cord segments were usually estimated by means of their relations to dural root exits, this relationship having been based on a number of dissections of normal cords.

After proper hardening, the cord was cut transversely into a number of pieces without attempting to remove the dura, so as not to disturb the rootlets within. The exact location of each of these cuts in relation to dural root exits was indicated on the sketch. The pieces were cut 2 to 3 mm. thick and imbedded in celloidin. Sections from each piece were stained by at least three methods, Hematoxylin-eosin, Van Gieson and Weigert-Pal.

PART 1. CLINICAL IMPROVEMENT AFTER THE PRODUCTION OF THE LESION IN RELATION TO THE HISTOLOGICAL FINDINGS AT DIFFERENT STAGES

This portion of the research is based on the studies of twenty dogs and their spinal cords. The important points are summarized in table 1. Glancing at this, it is seen that after such a lesion as described, almost all the animals showed more or less clinical improvement. It is further seen that this improvement

TABLE 1

		Clinical improvement				Histological condition	
Laboratory number of dog	Number of days after lesion that autopsy was done	When first noticed	One month after lesion	Two months after lesion	Three months or more after lesion	Spinal cord	Roots and nerves
71	10	No improvement				Mass of débris including broken down myelin and blood, which is entered by granulation tissue from pia and dura. This granulation tissue more dense toward periphery. In the interior there are young fibro blasts with elongated nuclei. Also many new capillaries. Compound granular cells, small round cells and leucocytes also present. Areas of beginning necrosis in neighboring grey matter.	Marked degeneration in some of the fibers
90	14	Distinct improvement noted on 5th day				Similar to above.	Similar to above
77	31	Distinct improvement noted on 5th day	Improvement			Marked formation of cavity divided into compartments. Granulation tissue denser than above. Many myelin droplets left. Some fibroblasts and compound granular cells. Less débris than in above. Leucocytes and round cells in new connect. tissue. Little repair in grey matter. Some ant. horn cells swollen and pale.	Similar to above
100	33	Distinct improvement noted on 6th day	Improvement			Similar to above.	Similar to above
76	36	Distinct improvement noted on 11th day	Improvement			Similar to above.	Complete or almost complete degen. of some of the fibers
66	50	Distinct improvement noted on 9th day	Improvement			Similar to above.	Similar to above
72	60	No improvement	No change	No change		Dense scar with small cavities adjoining. These contain a little débris and a few myelin drops small in size. Some small nerve fibers, perhaps new, entering cavities. Many round cells.	Similar to above
61	63	Distinct improvement noted on 9th day	No change	No change		Zone of dense scar from dura. Next a zone of less well organized connect. tissue containing in its meshes compound granular cells and myelin drops. Next a zone of a relatively few fibro blasts and myelin drops, débris in large amounts and old blood. Finally normal cord substance. Some ant. horn cells are bloated.	Some remains of broken down myelin in fibers
47	78	Gradual improvement	Improvement	No change		One main large cavity, which is lined by thin layer of new connect. tissue. Ant. horn cells show bloating and chromatolysis.	Some beginning regeneration
42	112	Gradual improvement	Improvement	Improvement	Slight improvement	Dense scar with large patches of round cells. Many leucocytes and new vessels.	Some fibers show signs of degeneration. Others suggest new regeneration
86	159	Gradual improvement	Improvement	Improvement	Slight improvement	Much dense scar. Two large cavities still containing the remains of myelin in small amounts. Distortion of grey matter. Ant. horn cells often bloated and show chromatolysis.	Similar to above
78	178	Distinct improvement	Improvement	Slight improvement	No change	Much dense scar. Large cavity, which tends to follow outline of grey matter. Distortion of grey matter. Vacuolation of ant. horn cells.	Increase in connect. tissue in nerve trunks
75	222	Distinct improvement noted on 13th day	Improvement	Improvement	No change	Much dense scar containing cavities. Ant. horn cells pale and bloated, having lost their polygonal shapes.	Similar to above
23	252	Gradual improvement	Improvement	Improvement	No change	Dense scar and cavities. Broken down tissue in grey matter. Vacuolation of ant. horn cells.	Similar to above
68	258	Gradual improvement	Improvement	Improvement	No change	Dense scar containing cavities.	Signs of new regeneration in roots
70	266	Gradual improvement	Improvement	Slight improvement	Slight improvement	Dense scar, débris and light connective tissue. Cavities. Distortion of grey matter.	Good regeneration of many fibers
73	275	Distinct improvement noted on 6th day	Improvement	Slight improvement	No change	Light connect. tissue and areas of partial necrosis in grey matter. Some dense scar with cavities.	Similar to above
69	277	Distinct improvement noted on 6th day	Improvement	Slight improvement	No change	2 large cavities, one of which follows outlines of grey matter. Dense scar.	Similar to above
64	292	Gradual improvement	Improvement	Improvement	Slight improvement	1 large cavity. Thick scar in dura.	Similar to above
65	319	Gradual improvement	Improvement	Slight improvement	Slight improvement	Dense and light scar, one cavity, débris and remains of myelin. Areas of necrosis in grey matter. Ant. horn cells often swollen, bloated and pale.	Similar to above

fell into two stages, (1) a sudden improvement occurring in the first or second week, and (2) a slow improvement continuing for three or four months.

Character of the clinical improvement

The paralysis immediately following the operation was often a complete paraplegia, sometimes of one leg only, and rarely of the tail and sphincters. The dog lay in the kennel, seemingly stunned and not caring for food. He appeared feverish and thirsty. After a day or two, in spite of the paralysis, he made some attempt to get over the ground, with but partial success. The sudden improvement in the first or second week was often very marked, a paraplegia sometimes changing into a mere turning of one paw. Figs. 2, 3, 4 and 5 show characteristic changes in two of the dogs. Whether or not the improvement began suddenly, there was always a period of gradual recovery ending in a permanent state of residual paralysis.

The question is, how much of the improvement is based on changes which are histologically demonstrable? The table (table 1) indicates what the chief histological changes were (the topography of ten of the lesions is studied in Part 2).

Histological summary

In the early autopsies the location of the lesion was evidenced by a mass of débris, including broken down myelin in drops, leucocytes and round cells in clumps. A mass of granulation tissue had formed along the dura, at the place of injury. This entered the mass of débris. In this stage, cavities were not apparent, but in the neighboring grey matter were small areas of beginning necrosis.

In animals autopsied at the end of one month, the most noteworthy thing was the appearance of cavity-like spaces. These were formed of connective tissue bands some of which had become well defined owing to the partial removal of the tissue débris. By this time the scar connected with the dura had become quite dense.

At the end of two to three months, the sections showed the permanent histological characteristics of the lesion. These were either cavity spaces or scar, or both. The cavity spaces were sometimes sharply circumscribed, and occasionally their outlines corresponded to the original outlines of the grey matter. In general, it seemed that the amount of scar depended on the amount of direct injury to the pia and dura, and the size of the cavity-spaces on the extent of the original destruction of cord substance, although of course, the extent of the original cavities could not be completely judged on account of tissue repair. In some places the grey matter showed marked distortion of outline, suggesting contraction.

As to signs of nerve regeneration in the cord, one occasionally saw among the débris and granulation tissue small fibers with faint coats of myelin. But of course, there was no way of telling whether these indicated repair; if they did it could have been slight only.

In all stages, one saw striking changes in the ganglion cells of the gray matter, near the lesion. These consisted in alterations in size, shape and staining reactions as well as chromatolysis and vacuolation.

Inferences from the above

Judging from the histological condition one could divide the anatomical appearances into two stages, roughly corresponding to the two stages in the clinical improvement. It seemed likely that the early improvement was partially due to the removal of tissue débris, because the cavities became apparent about that time. Also as shown clinically, by the swelling about the wound, there was at first marked fluid exudate, the absorption or escape of which was undoubtedly a factor in that improvement.

As to the more gradual improvement that supervened in most of the cases, disregarding the anatomic evidence of repair of nerve tissue in the cord itself, as being too slight to explain the marked restoration of function, there are four other explanations to be considered: (1), actual regeneration of nerve fibers in roots and peripheral nerves; (2), vicarious activity of nerves and muscles

(including employment of other paths in the cord); (3), failure of many of the neurones originally pressed on by the exudate to degenerate completely, so that with removal of the exudate they recovered their functions; (4), changes in subjective state in the animal as brought about by lessened discomfort. (The last two explanations might also apply to the early improvement.) We cannot say which of these factors were most important—in fact, it is possible that all of them had a share in explaining the clinical change for the better.

Previous researches on the subject

That marked restoration of power may occur after lesions in the central nervous system is well known. This applies both to cortical extirpations,[2] and to hemisections in the cord.[3] The fact that there is also considerable restoration of power after complete section would seem to indicate that there might be regeneration across the scar. This has been actually observed in some of the lower animals.[4] Brown-Sequard noted it in fishes and Fraisse in amphibians. But in higher animals it has not been observed,[5] and for this reason, in cases of hemisection restoration of the clinical state has been said to be partially due to the employment of paths on the contralateral side. Be that as it may, it is not necessary to base the restoration of function on nerve repair across the scar because the clinical improvement seemed practically as great in our cases where, on account of the extent of the lesion, conditions for nerve repair were not favorable and where, moreover, signs of such repair were scarcely to be made out.

Bearing of above inferences on infantile paralysis

The spontaneous restoration of power noted in our dogs is very similar to that often observed in infantile paralysis. This applies both to the early and late improvement. The former,

[2] Sherrington: Integrative action of the nervous system, 1906, p. 277.
[3] Weiss: Sitz. d. Akad. d. k., Wissensch., Wien, 1879, Bd. 80.
[4] Bechterew: Functionen der Nerven Centra, 1908, vol. 1, p. 652.
[5] Schiefferdecker: Virchow's Archiv, Bd. 67.

also in that disease, is at least partially due to the removal of inflammatory material, somewhat as in the conditions described above, where the exudate is dependent only on simple mechanical lesions, although part of the recovery in infantile paralysis may be related to cell changes which take place as a result of the expulsion or neutralization of specific toxines. As regards the later improvement in that disease, one is just as much in doubt as to which of the factors above mentioned is of greatest significance.

It is plain, however, that the spontaneous recovery observed in infantile paralysis is perhaps an attribute less characteristic of the disease than of the anatomical structures which the disease attacks. Whether it is a clean section, or a crushing lesion or an inflammation set up by some virus, there are certain succeeding manifestations that seem to be common to all these antecedents, and these manifestations are exhibited with striking clearness simply because of the fact that in the central nervous system, a relatively small anatomical change affects an extremely large physiological sphere.

PART 2. THE LOCALIZATION OF CENTERS IN THE LUMBO-SACRAL CORD

Method of study and reasoning

This part of the research is based on ten of the experiments. The study of data furnished by this material is summarized in tabular form (table 2) and each experiment is illustrated with a photograph, and a diagram based on the sketch made at the autopsy. In these diagrams only direct involvements due to the lesions are indicated. The degenerations were partly due to secondary operations, and are therefore best omitted, because under the circumstances they throw no light on the localization.

In handling the data, each case is analyzed for itself and the inferences individually derived are collated according to the important clinical and physiological headings under which each was studied. The cord segments were numbered according to the roots, calling the root issuing beneath the last rib, the 13th.

TABLE 2

NUMBER OF EXPERIMENT	CONTROL OF JOINTS	RESPONSE OF LEG TO POINT	RESPONSE OF PAW TO HEAT (PAIN)	REFLEXES		SPHINCTERS		CORD AND ROOT INVOLVEMENT (See diagrams)	POSITIVE CONCLUSIONS FOR INDIVIDUAL
				Knee jerk	Anal	Anus	Bladder		
64	Paralysis of dorsal flex. of left paw and ankle Direct stimulation evoked no response in left E. P.	Poor on both legs	No test	Absent on left	Sluggish	N	N	In upper 7th L. seg. post. horns and cols. on both sides. On left, lat. col. and upper $\frac{2}{3}$ of ant. horn. In 1st S. seg. cent. part of grey matter on left. In 5th, 6th, 7th and 1st S. seg. postero-mesial col. 5th and 6th post. roots.	Dorsal flexion of paw and ankle (E. P.), in dorsal part of ant. horn of upper 7th L.
78	Paralysis of dorsal flexion of right paw and ankle. Weak extension of rt. hip and knee Direct stimulation evoked no response in right E. P.	Poor on rt. leg	Poor on right	Absent on right	Sluggish	N	N	In mid. 4th L. seg., lower part of rt. ant. horn and adjoining col. In high 5th L. seg., entire rt. ant. horn and adjoining col. In low. 5th L. seg., entire rt. half of cord except ant. mesial col. In mid. 6th L. seg., whole cord except ant. cols. and lower part of lt. lat. In upper 7th rt. ventro-mesial col. very narrow 4th, 5th and 6th rt. ant. roots.	Dorsal flexion of paw and ankle (E. P.)—in lower 6th L. I. P. control lower (in 7th) Extens. of hip and knee in 5th Gap between centers of lower and upper leg, perhaps containing centers of hamstrings
86	Paralysis of rt. paw and ankle and of dorsal flex. of left paw and ankle. Weak extension of rt. hip and knee and some weakness of lt. hip and knee Direct stimulation evoked no response in either E. P. and very slight (of toes only) in rt. I. P.	Poor on rt. leg	Poor on right	Absent on right Weak on left	Sluggish	N	Weak	In upper 5th L. seg., rt. side of cord except pyram. tract, also lt. ant. horn. In low. 5th L. seg. rt. side of cord. In mid. 6th whole cord except lt. ant. col. and small part of lt. ant. horn. In upper 7th inner $\frac{2}{3}$ of rt. ant. horn. 5th and part of 6th rt. and lt. ant. and post. roots and 4th rt. ant. root.	Dorsal flex. of paw and ankle (E. P.) in mid. 6th Plantar flex. of paw and ankle (I. P.) in inner dorsal part of ant. horn of upper 7th Extens. of hip and knee in 5th
69	Slight weakness in dorsal flex. of rt. paw and ankle	N	N	N	Sluggish	N	N	In mid. 6th all of grey matter and dorsal and ventral cols. which are partly spared on left. In upper 7th rt. ant. horn, rt. lat. col., both ant. cols. and lower $\frac{2}{3}$ of lt. ant. horn. 6th rt. post. and 6th and 7th rt. ant. roots.	Dorsal flex. of paw and ankle (E. P.) partly in upper 7th. Gap between centers of lower and upper leg
65	Paralysis of entire left leg except for flex. of hip. Weak extension of knee and weak flex. of hip on right	Poor on left	No test	Absent on left Weak on right	Sluggish	N	N	In upper 6th whole left of cord, and dorso- and ventro-mesial cols. and inner part of grey matter on rt. In low. 6th lt. ant. horn and lt. antero-lat. col.; also inner part of rt. ant. horn. In low. 7th outer part of lt. ant. horn and lt. antero-lat. col. In 2d Sac. grey matter about central canal. 5th 6th, 7th and 1st lt. ant. roots; 5th and 6th lt. post. roots; 6th rt. ant. root.	Control of most of leg in 5th seg. and those below Point sensation passes through 5th post. root
75	Weakness of gluteals and hamstrings on rt. hip flexed and knee straight and stiff Paw paralyzed and flaccid	Poor on right	Poor on right	Absent on right	N	N	N	In mid. 6th dorsal cols. and inner part of base of rt. post. horn. In upper 7th rt. side of cord and dorsal col. and inner part of grey matter on lt. In 1st Sac. rt. side of cord and inner part of lt. ant. horn. 6th and 7th rt. post. horns.	Sciatic centers in 7th L. and 1st Sac.
70	Spastic loss of control of hind parts from pelvis down. Weakness of erector spinae, glutei and quadriceps. Hamstrings fairly good	Poor on left	N	N	N	N	N	In mid. 4th rt. ant. horn. In upper 5th lt. post. and all of antero-mesial cols. rt. ant. horn, most of lt. grey matter. In mid. 5th all of cord except rt. post. horn. In low. 5th whole cord. In high 6th lt. of cord. In mid. 6th ant. cols. and lower part of ant. horns. 4th, 5th, and part of 6th rt. and lt. ant. roots 4th lt. post. root.	Some crural and gluteal centers in upper 5th. Hamstrings below mid. 6th Transection in lower 5th cuts off brain control of lower leg Point sensation passes through 4th post. root Heat-pain sensation above mid. 5th K.J. anal reflex, control of sphincters below upper 6th
72	Flaccid paralysis of hind parts from pelvis down.	Poor on both	No test	Absent on both	No test	Weak	Weak	In mid. 5th, base of dorsal cols. part of rt. ant. root. In low. 5th, all of grey matter and dorsal and vent. cols. except left post. horn and small part of vent. col. adjoining. In mid. 6th all of grey matter and rt. ventral and lat. cols. In mid. 7th rt. ant. horn and inner part of lt. grey matter. 5th, 6th and part of 7th rt. lt. ant. roots, rt. 5th post. root.	Control of hind parts including sensation, K.J.'s and sphincters in 5th to 7th inclusive
68	Paralysis of tail and slight weakness in dorsal flex. of rt. paw.	Poor on tail On legs N	Poor on tail	N	Sluggish	Weak	Weak	In mid. 6th dorso-mesial col. In mid. 7th rt. side of cord, dorso- and ventro-mesial cols. and inner part of lt. grey matter. In low 1st Sac., whole of cord (conus). 5th and 6th rt. ant. and post. roots.	Control of motion and sensation to point and heat-pain in tail in 7th L. and 1st S. Anal reflex and control of sphincters in 7th L. and 1st Sac.
73	Paralysis of tail	Poor on tail	Poor on tail	N	Sluggish	N	Weak	In upper 7th, all of grey matter except lt. post. horn; also vent. cols. and lower part of rt. lat. col. and rt. dorsal col. In lower 7th entire cord (conus).	Control of motion and sensation to point and heat-pain in tail in 7th L. Anal reflex and control of bladder in 7th L.

Letter N = normal. E. P. = external popliteal. I. P. = internal popliteal

Unfortunately, the ribs themselves were not counted, so that the possibility of variation must not be lost sight of, as a source of error.

The most pertinent information is that obtained in the individual case by comparing rights and lefts. Other things being equal, one may in certain cases, ascribe a unilateral effect to a corresponding unilateral lesion. In the same way by proper elimination, bilateral effects may sometimes be ascribed to bilateral lesions. It will be apparent that the collation of all the evidence according to numerical segments offers less exact conclusions than those which can be derived in the same individual and more especially with reference to the relative position of centers. But by comparing relationships and detecting correspondence, certain inferences of significance may be obtained. Aside from the assumption that the upper leg centers are, generally speaking, higher than those of the lower leg, no further assumptions were used, except, of course, such as are based on the classical conceptions of the functions of anterior and posterior roots and of the white and grey matter of the spinal cord.

Inferences from individual experiments: summary

Experiment 64. (Figs. 6 and 7.) External popliteal paralysis on left not accounted for by slight anterior root damage as there was similar damage on right where there was no paralysis. Therefore the external popliteal centers must be in the upper 7th lumbar segment, and by comparing right and left anterior horns, they must be in the dorsal two-thirds of the horn. Loss of left knee jerk accounted for by involvement of 5th and 6th posterior roots. If internal popliteal centers are at the level of or below the external popliteal centers (see Experiments 78 and 86), paths for voluntary control seem to run in the anterior column.

Experiment 78. (Figs. 8 and 9.) Weakness of right knee and hip best explained by involvement in 5th lumbar segment and anterior root filaments attached. As lesion in grey matter of middle 6th lumbar leg is bilateral and left leg seemed good, it could not account for paralysis of right external popliteal, which is consequently traceable to damage of right anterior roots attached to lower 6th and upper 7th lumbar segments. Both internal popliteals being good, their centers must be lower than external popliteal centers, for they could not be higher on account of bilateral lesion just above, which lesion also suggests a possible gap between upper and lower leg centers. Extinction of right knee jerk explained by 5th and 6th anterior root involvement. Voluntary dor-

sal and plantar flexion of left paw and ankle, and plantar flexion of right seems possible with only anterior columns open.

Experiment 86. (Figs. 10 and 11.) External popliteal paralysis on both sides due to damage in middle 6th and lumbar segments and roots attached. Almost complete paralysis of internal popliteal on right and not on left corresponds to lesion in upper 7th lumbar segment. Loss of point and heat-pain sensation on right and not on left explained by lesion in right half of cord in 5th lumbar segment. The difference in damage to grey matter in lower 6th and upper 7th lumbar segments must account for preservation of left knee jerk. As internal popliteal is good on left and its centers are in upper 7th lumbar segment, impulses from the brain to its synapses must have passed through the marginal portion of the anterior column in the segment above.

Experiment 69. (Figs. 12 and 13.) The difference between the right and left anterior horn involvement in the upper 7th might explain the weakness of the right external popliteal. Lesion in 6th lumbar segment suggests a gap between upper and lower leg centers. This lesion seems to have had no effect on either knee jerk or on sensations, suggesting that these latter must have entered through upper 6th rootlets or higher. As the centers presiding over control of right paw may be presumed to be below the 5th lumbar segment, the extent of the lesion might denote that such control was cut off on that side. Therefore, as the dog used the paw quite well (except for the external popliteal weakness mentioned) it is possible that impulses crossed from the other side, where the lateral column was good.

Experiment 65. (Figs. 14 and 15.) The extensive paralysis of the left leg and paw together with loss of knee jerk, best accounted for by severe root involvement, while the weakness in the right upper leg is accounted for by damage to anterior horn in lower 6th lumbar segment and some of the 6th anterior root filaments.

Experiment 75. (Figs. 16 and 17.) Here permanent flexure of right hip and stiffness of knee, perhaps due to weakness of gluteals and hamstrings respectively, caused by damage of 5th, 6th and 7th anterior roots. The lower leg paralysis explained by lesion in 7th lumbar and 1st sacral segments. Loss of right knee jerk explained by damage to 6th posterior root.

Experiment 70. (Figs. 18 and 19.) The lesion in lower part of 5th lumbar segment involved entire cord, practically acting as a trans-section. This accounts for loss of control and spasticity of hind parts, and places most of leg centers below that segment. It is likely that preservation of knee jerk, anal reflex and control of sphincters is due to sparing of centers below middle 6th lumbar segment (the anterior roots being also destroyed above that level).

Experiment 72. (Figs. 20 and 21.) The lesion in upper 5th, 6th and 7th lumbar segments, with the corresponding anterior root damage accounts for loss of control of hind parts, including point sensation, knee jerks and sphincters.

Experiment 68. (Figs. 22 and 23.) Impairment of control of motion, point and heat-pain sensation of tail, together with impairment of anal

reflex and sphincteric control, all accounted for by lesion in 7th lumbar and 1st sacral segments. Slight paralysis of right paw also explained by damage to right anterior roots.

Experiment 73. (Figs. 24 and 25.) Impairment of control of motion, point and heat-pain sensation in tail, together with impairment of anal reflex and bladder control, all accounted for by lesion in the 7th lumbar segment.

Correlation of data derived from individual experiments

A. Control of joints. Upper leg centers are mostly in the 5th and 6th lumbar segments, that is, about the level of the 4th dural root exit. Lower leg centers are in the lower 6th and the 7th lumbar segments, that is, at the level of the 5th dural root exits. Nuclei of the external popliteal, the internal popliteal and the tail are somewhat circumscribed and relatively isolated, and the nucleus of the external popliteal is, as a whole, higher than that of the internal popliteal. There may be some overlapping of nuclei, but in one portion of the cord, namely, the middle 6th lumbar segment, there seem to be relatively few centers, the nuclei of the upper and lower leg seeming to be respectively above and below this apparent gap. (Possibly the hamstring centers lie here.)

It is suggested that the anterior columns contain fibers from the brain which convey volitional impulses.

B. Knee jerks. Its extinction or weakening is consistent with corresponding damage to either the roots or grey matter of the 5th and 6th lumbar segments.

C. Sensation. As to point sensation of the whole leg, the 4th and 5th posterior roots seem important links in the afferent chain.

As to heat-pain sensation of the paw (studied by immersion in hot water), the test was omitted in three of the experiments and was always accompanied by loss of point sensation. In one case however (Experiment 70), point sensation was lost without corresponding impairment of heat-pain sensation in paw.

D. Anal reflex. This test was omitted in one case, found impaired in seven and normal in two.

E. Control of sphincters. Their centers lie below the 6th lumbar segment. It is suggested by comparison of two cases

(Experiments 68 and 73) that the bladder centers are higher than the anal centers. Sphincteric centers are very close to tail centers (5th dural exit).

F. Conclusion. Inferences cannot be drawn for every point investigated, but there is significance to certain isolated facts, such for example, as pertains to the relative isolation of the external popliteal and internal popliteal centers, suggesting perhaps that these nerves, which on account of their individual spheres of distribution control the best coördinated joint movements, have their nuclei relatively best defined. The main suggestion is that the grouping of cells seems to correspond at least roughly to the gathering of fibers in individual peripheral nerves.

Comparison of above inferences with current views on the subject

As regards the cases where preservation of voluntary control in certain muscles seemed to depend on paths in the anterior columns, which alone were open, other experiments are on record,[6] which show that in dogs and other animals, these columns do convey motor fibers from the brain. There is also the possibility of the employment of paths on the contralateral side (cf. Part 1) and in one of our cases (Experiment 69) this seems to be the only explanation for preservation of control of the paw, for the lesion had blocked all the homolateral paths higher up.

With reference to the knee jerk our localization in the 5th and 6th lumbar segments corresponds to Sherrington's more accurate findings.[7]

As to sensations, anal reflex and relative warmth of the paws, our findings are too few to be entitled to colligation with those of others.

The most important question that we have to consider is the significance of the cell-groups in the grey matter of the cord. Three methods have been previously used to investigate this point, the first being that of direct stimulation of spinal roots. The findings depend on the presumption that the cells of origin

[6] Bechterew: Functionen der Nerven Centra, 1908, vol. 2, p. 667.

[7] Sherrington: Schaefer's Text-book of Physiology, 1900, vol. 2, p. 874.

of the fibers in a given root are about on a level with the superficial origin of that root from the cord. Sherrington[8] has advanced some evidence for this, which evidence is based partly on the fact that after section through the cord just above a given anterior root, very little degeneration is to be seen in the fibers of that root, and partly on the results of direct stimulation of roots above and below the place of section. Besides Sherrington's contribution important reports on the results of direct stimulation of roots have been made by Bikeles and Gizelt,[9] Langley,[10] and Risien Russel.[11] The subject has been studied in connection with the formation of the lumbo-sacral plexus. The results agree fairly well, the fibers constituting the main nerve trunks being said to arise from the cord in the following descending order: crural, obturator, gluteal, sciatic, tail and sphincters. The internal popliteal fibers are, as a whole, placed higher than the external popliteal. There is supposed to be considerable overlapping of nuclei. Further than this longitudinal relationship, little information is obtainable by the method. Our results conform fairly well to the above order except as regards to relative height of the external popliteal and internal popliteal.

A second method is based upon the pathological findings in human beings in such conditions where definite motor paralysis were clinically under observation[12] (infantile paralysis, tumors of the cord, etc.). The observations are, however, very fragmentary.

The third method consists either in the amputation of limbs or parts of limbs, or in the excision of peripheral nerves, and later studying the ganglion-cell changes to be observed in the spinal cord. Valuable contributions have been those by Sano,[13] Van Gehuchten and Nelis,[14] Flatau,[15] Marinesco,[16] Bruce,[17] and

[8] Sherrington: Journ. of Physiol., vol. 13, 1892, p. 621.

[9] Bikeles and Gizelt: Pflüger's Archiv, 1905, vol. 106, p. 43.

[10] Langley: Journ. of Physiol., 1891, vol. 12, p. 347.

[11] Risien Russel: Proceed. Royal Soc., 1894, vol. 54, p. 243.

[12] Wickmann: Die Rückenmark-nerven und ihre Segment-bezüge, Berlin, 1901.

[13] Sano: Les localizations des functions motrices de la moelle épinière, 1908.

[14] Van Gehuchten and Nelis: Jour. de. Neurol., 1898, p. 301.

[15] Flatau: Archiv f. Anat. u. Physiol. Physiol. Abt., 1898, p. 112.

[16] Marinesco: Revue Neurol., 1898, p. 483.

[17] Bruce: Topographical atlas of spinal cord. 1901.

Knape.[18] Knape is practically the only one of these who leans toward the theory that cell groups represent collections of fibers in peripheral nerves. Even he does not describe sharply circumscribed nuclei as representing these nerves. He let his animals run a long time after excising the nerves, and before he studied the cords, in several cases allowing an interval of almost five years to intervene. His nuclei extend over more segments than our own.

As to previous observations on sphincteric control, reliance has usually been placed upon the stimulation of roots.[19] According to most observers[20, 21, 22] the nerve supply affecting control of micturition in dog and cat comes from two sources in the cord, an upper from the 3d, 4th and 5th lumbar roots, and a lower from the 2d and 3d sacral roots. Nerve fibers are sorted out in the hypogastric plexus before they finally pass to the bladder itself. According to Bechterew,[23] Sherrington,[24] Langley and Anderson,[25] and others, the lower source is especially important. This does not conform to our localization for bladder control in the 7th lumbar or 1st sacral segments.

Very much like the bladder, the rectum receives its nerve-supply from two sources,[26] and from about the same spinal nerves. Moreover, as in the case of that organ, the sacral nerves are much more important than the lumbar. Masius,[27] and Ott,[28] like ourselves, place the center higher than the lower source given by the others.

[18] Knape: Ziegler's Beiträge. 1901, vol. 29, p. 251.

[19] Langendorff: Nagels Handbuch der Physiologie, vol. 4, 1st half, p. 350.

[20] Nawrocki and Scabitschewsky: Pflüger's Archiv, 1891, vol. 48, p. 335, vol. 49, p. 141.

[21] Budge: Zeitsch. f. rational med., 1864, vol. 21, pp. 1 and 174.

[22] C. C. Stewart: Amer. Jour. of Physiol., 1899, vol. 2, p. 182.

[23] Bechterew: Functionen der Nerven Centra, 1908, vol. 1, p. 292.

[24] Sherrington: Schaefer's Text-book of Physiology, 1900, vol. 2, p. 874.

[25] Langley and Anderson: Jour. of Physiol., 1895, vol. 19, p. 71.

[26] Starling: Schaefer's Text-book of Physiology, 1900, vol. 2, p. 336.

[27] Masius: Bull. Acad. Royal de Belgique, pp. 67, 68.

[28] Ott: Jour. of Physiol., 1879, vol. 2, p. 54.

The significance of cell-groups in the grey matter of the spinal cord

According to different authors, the cell-groups in the gray matter of the cord are variously supposed to represent muscles, peripheral nerves, primary metameres or movements (as in the cortex). After a rather careful scrutiny of some of the literature, beside that mentioned above, we feel that in the present state of our knowledge, there is not sufficient evidence for any of these explanations. It is by no means certain that these cell-groups have any physiologic significance whatever. According to Knape, as above shown, and according to our own experiments, the findings seemed to point somewhat toward the peripheral nerve theory. Another piece of evidence for this theory is the fact that some of the cranial nerves have their cells of origin grouped in fairly well circumscribed nuclei. But neither is this analogy, nor the other evidence which we have cited, of sufficient weight to carry conviction. If the grouping of cells in the cord is ever susceptible of explanation, much further investigation will be required.

PART 3. NERVE FUSION ATTEMPTED IN THE CAUDAS AND THE SCIATIC NERVES IN ANIMALS PARALYZED BY MECHANICAL LESIONS IN THE SPINAL CORD

This part of the research is based on eight cases, the only ones in which the animals survived both the original spinal lesion and a secondary 'fusion' done some time after they had reached permanent states in their paralyses.

The aim of the procedure

Nerve fusion, it has been stated, consists simply in uniting two or more nerves by tying them together with absorbable ligatures. In the preliminary report[29] the theoretical basis for this procedure is given, the important point being that the direction of fibers regenerating in scar is governed by conditions offered by the mass of proliferated cells and nuclei which are here formed. As these are laid down in all directions the new fibers which develop in the interstices of the cells must grow accordingly. Therefore it

[29] Feiss: Boston Medical and Surgical Journ., May 11, 1911.

may be hoped that permanent changes in nerve pattern might result, and if two or more nerves are joined, that the mechanical attributes of the scar might cause fibers from fascicles of one nerve to pass into those of another. Besides, as shown by Perroncito,[30] Bethe[31] and others, one might even hope for branching of some of the regenerating fibers, so that if certain tracts, previously emptied by the paralysis, are entered by the new branches, there might result not only a change in nerve pattern but perhaps also a relative increase in the number of fibers. The purpose of the ligature is thus seen—it not only brings the nerves into physical apposition, but it also crushes them so as to cause the scar to form. Being absorbable (cat-gut) it disappears of itself. Compared with the older method of nerve crossing by suture, the theoretical advantages are: (1), that no division of nerves may be necessary; (2), that as many nerves as are in physical proximity may be included in the fusion; and (3), that change of nerve pattern may be hoped for in the individual nerve, even if no other unites with it.

Of special interest is the fact that in the cauda, at the region of the interspace between the dural exits of the 5th and 6th lumbar roots (dog) one may intradurally gather all the roots which supply the hind limb and tail into a compact bundle, and fuse them in the manner suggested above. In fact, by retracting the sensory roots, the motor roots alone may be thus joined together.

Below are given summaries of experiments in which fusions were attempted either in the cauda or in the sciatic nerves, some months after primary lesions were produced. These lesions have already been described (Part 2). The essential facts in the secondary fusions are given in table 3.

Experimental data[32]

Experiment 64. Lesion, January 18, 1910. On April 27, 1910, residual paralysis (fig. 6) chiefly of left external popliteal. On this date, following operation, left sciatic exposed, and after faradic stimulation[33]

[30] Perroncito: Ziegler's Beiträge, 1907, vol. 42, p. 354.

[31] Bethe: Pflüger's Archiv, 1907, vol. 116, p. 385.

[32] All exposures of roots and nerves were made under full ether anaesthesia.

[33] In all the experiments a du Bois coil with 10,000 windings of the secondary and a two-pint Daniell cell in the primary current, were used.

determining that there was no response in the external popliteal the two popliteals (sciatic) crushed with haemostat and tied together with three cat-gut ligatures one-quarter inch apart. On August 19 (114 days after fusion), same nerves exposed, and firm neuroma found at place of fusion. Faradic stimulation of external popliteal showed flexion of toes, but no extension. Internal popliteal responded normally. On November 6 (193 days after fusion) similar responses and animal sacrificed. No clinical improvement noted.

TABLE 3

Number of experiment	Distribution of paralysis	Fusion: Number of days after date of lesion	Fusion: Kind of fusion	Results of fusion: Clinical (functional)	Results of fusion: Physiologic (faradic)	Results of fusion: Anatomic (myelination)
64	Paw and ankle E. P.	97	Popliteals	Slight (?) improvement	Negative	Some regeneration in and below neuroma
69	Paw and ankle E. P.	123	Popliteals	Worse than before	Negative—Stumps not united	Some regeneration in peripheral stump
78	Paw and ankle E. P.	129	Popliteals	No improvement (death in 60 days)	No tests	Beginning regeneration in neuroma. None in E. P. below neuroma.
65	Most of leg	99	Ant. roots in cauda	As before	Doubtful	Slight regeneration
70	Most of leg	124	Ant. roots in cauda	Worse than before	Responses in roots and nerves	Fair regeneration below neuroma
75	Most of leg	122	Ant. roots in cauda	Worse than before	No tests	Slight regeneration below neuroma
68	Tail	85	Cauda-Sacral and coccygeal roots	Fair improvement	Good responses	Some regeneration in and below neuroma
73	Tail	111	Cauda-Sacral and coccygeal roots	Fair improvement	Fair responses	Some regeneration in and below neuroma

Anatomic report (In this and the following experiments, sections were stained by Weigert-Pal and general methods): Left sciatic above scar showed normal fibers and some unusual patches of connective tissue. Sections through midst of neuroma showed many small, and partially myelinated fibers some in bundles and others scattered among the cells of the scar. Scar dense and contains numerous nuclei. Popliteals below scar show scattered fibers with large interspaces and myelination, although not complete further advanced than in scar. Some large areas where no fibers appear suggesting that sheaths emptied by lesion, have not been filled.

Experiment 65. Lesion, January 19, 1910. On April 28, 1910, residual paralysis (fig. 14) chiefly of left leg. On this date, following operation: 6th and 7th arches removed, dura opened, cauda exposed. Sensory roots lifted aside and such motor roots as lay in the field stimulated. No response except in tail. Motor roots fused with one cat-gut ligature and crushed with haemostat. Dura sewed. Wound closed tight. No clinical improvement noted after operation. On September 26, 1910, stimulation of roots attempted with doubtful results. Animal sacrificed.

Anatomic report: Partial disappearance of anterior roots at region of fusion. Some scar with only a few nerve fibers interwoven in it. Roots peripheral to scar show greater numbers of fibers and myelination further advanced.

Experiment 68. Lesion, March 17, 1910. On June 10, 1910, residual paralysis (fig. 22) chiefly of tail and slight weakness of dorsal flexion of right paw. On this date following operation: 6th lumbar arch removed and all sacral and coccygeal roots (both anterior and posterior) constituting cauda at this region, fused with two cat-gut ligatures. No change noted after operation till in September or October, when tail seemed to be moved better (figs. 26 and 27). Thereafter but little gain. On November 30, 1910 (173 days after fusion), all roots divided and stimulated peripheral to fusion-neuroma and found to evoke tail movements. Animal sacrificed.

Anatomic report: Place of fusion shows dense and knotty scar with large numbers of cells running in all directions. Among these, small and partially myelinated nerve fibers have formed. Roots peripheral to scar contain fibers better myelinated, some almost normal.

Experiment 69. Lesion, March 17, 1910. On July 18, residual partial paralysis (fig. 12) of muscles supplied by right external popliteal. On this date following operation: right sciatic divided above bifurcation and immediately resutured. Then popliteals fused by usual method with two cat-gut ligatures. (The object of the division was to promote fibrillation at the cut end of the central stump, before the fibrils entered the region of fusion.) Clinically dog became worse after the operation. On December 19, 1910, nerves investigated under ether and the cut ends of the sciatic were found ununited. Yet stimulation of popliteals below neuroma evoked responses. Animal sacrificed.

Anatomic report: Popliteals in fair states of regeneration.

Experiment 70. Lesion March 18, 1910. On July 20 residual spastic paralysis (fig. 18) of both legs. On this date operation similar to that done on dog 65. The immediate result was flaccidity of homolateral paw. December 8, 1910, all roots which were involved in the fusion scar divided and stimulated under ether. Leg movements evoked on side of fusion, similar to those on other side. Popliteals stimulated and responded to weak currents. Animal sacrificed.

Anatomic report: At region of fusion dense scar containing partially myelinated fibers. Peripheral to scar, roots show myelination better advanced.

Experiment 73. Lesion March 19, 1910. On July 8, 1910, residual paralysis (fig. 24) confined to tail. On this date operation similar to that done on dog 68. In September dog was wagging tail pretty well and seemed to have more strength in it. On December 20, 1910, all roots below fusion divided and stimulated, under ether, and tail responses evoked. Animal sacrificed.

Anatomic report: Region of fusion showed dense, knotty scar infiltrated with nuclei, also a few fascicles of good fibers and some partially myelinated fibers interwoven among the cells. At more caudal levels regeneration quite advanced.

Experiment 75. Lesion April 12, 1910. On July 12, residual spastic paralysis (fig. 16) of right leg, except for paw which was flaccid. On this date operation on right anterior roots similar to those done in dogs 65 and 70. Clinical result completely negative. Death by accident, November 20, 1910.

Anatomic report: In region of fusion, dense scar containing some faintly myelinated fibers.

Experiment 78. Lesion April 15, 1910. On August 22, 1910, residual paralysis (fig. 8) chiefly of right external popliteal. On this date following operation: external popliteal divided and fused to internal popliteal low down. Dog found dead, October 10, 1910.

Anatomic report: Right sciatic above fusion contained increased connective tissue spaces. At region of fusion dense scar with numerous nuclei. New nerve fibers among these running in all directions. Some regeneration in nerves below fusion.

Discussion

In the three dogs (Experiments 64, 78 and 69), in which the popliteals were fused, there was no functional gain, although there was anatomically some regeneration in all the nerves below the scar. In one of these (78) death occurred before any functional result could be expected. In dog 69 where the sciatic was sectioned higher up and the stumps failed to unite, the peripheral stump must have made new central connections through small fibers injured in the wound during the operation. The stim-

ulation tests in Experiment 64 could not be said to be positive for the external popliteal. As to the other five dogs, the three which had their anterior roots fused on one side could not be said to show any functional gain, although two (Experiments 65 and 70) showed some response to faradic stimulation in the roots below the fusion. Anatomically again, there was some regeneration.

The only animals in which functional improvement was suggested were dogs 68 and 73, both of which had paralyzed tails, and therefore had the fusion done so as to include all the roots, taking part in the innervation of that appendage.[34] In both of these, there also were signs of good regeneration, from the anatomical and physiological points of view. However, one could not be positive that the functional return of power was due to the fusion, because it took place so soon after. To help settle this point we performed similar operations in the cauda of three normal dogs. In these cases the tails were pretty well restored in power by the end of three months. It is likely that this early improvement is owing to the shortness of route between the nerve-collectors of the tail and the roots from which these are formed.

As regards the whole question of the fusion of nerves, it is not desirable, at the present time, to discuss it further on a basis of the experiments above described, but, at some future time, after becoming acquainted with conditions of regeneration after the fusion of normal nerves, it is likely that these experiments will be alluded to again.

In closing, I wish to acknowledge the assistance of Dr. R. H. Bishop in the conduct of many of the experiments, and of Dr. David Marine in the preparation and interpretation of anatomical material. I am especially indebted to Professor George N. Stewart, Director of the Laboratory. He has made many important suggestions in the plan and details of the work, as well as in the preparation of the manuscript.

Cleveland, Ohio.

[34] Schümacher: Anat. Hefte Beiträge zur Anat. und Entwickelungsgeschichte, 120 Heft., 1909.

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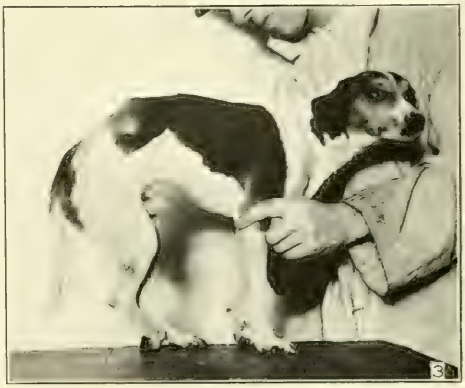

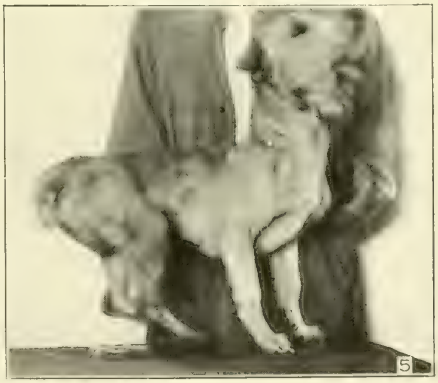

Fig. 1 Instrument used to produce lesions.
Fig. 2 Experiment 90; two days after lesion.
Fig. 3 Experiment 90; five days after lesion.
Fig. 4 Experiment 100; two days after lesion.
Fig. 5 Experiment 100; twenty-one days after lesion.

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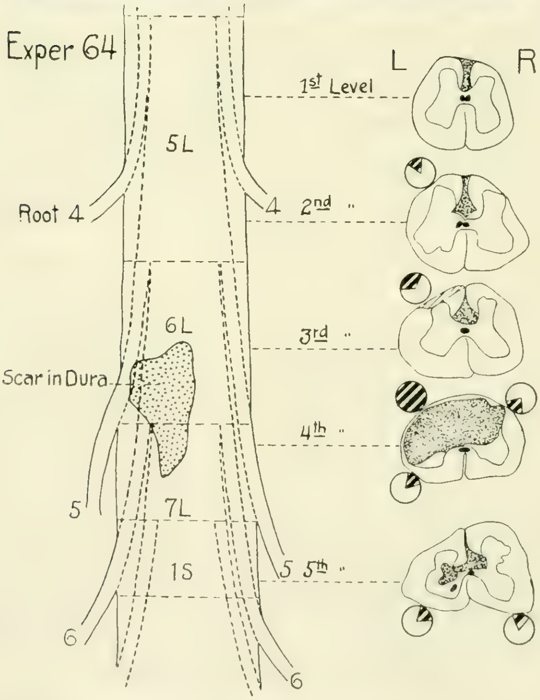

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Fig. 6 Experiment 64; ninety-four days after lesion. Left, no dorsal flexion of paw and ankle; direct stimulation of nerves showed *E. P.* practically all involved.

Fig. 7 In this and the following diagrams, the topography of the cord lesions is indicated; fine stipple represents cavity; parallel line shading represents broken down tissue; cross-hatching represents dense scar from dura; the heavy shading in the circles, which represent roots, indicates the extent of involvement at the level shown; degenerations are not represented. [E. P. and I. P. = internal and external popliteals respectively.]

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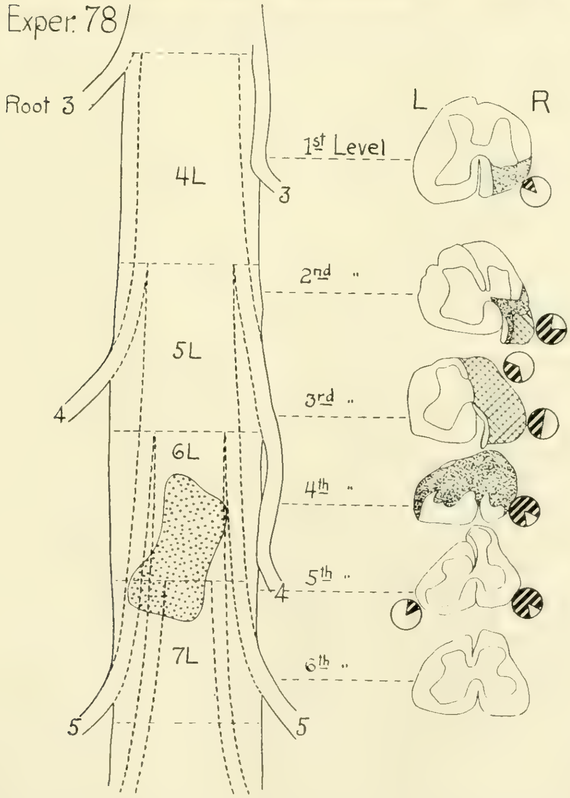

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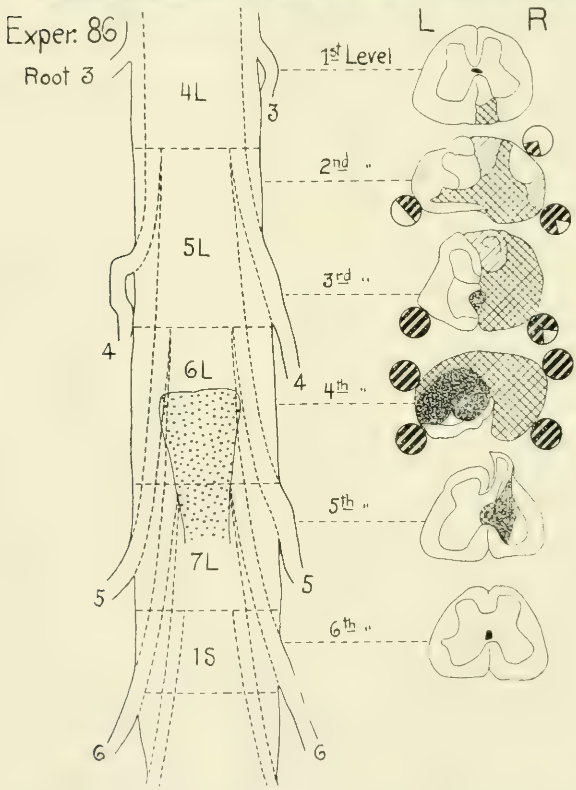
Figs. 8 and 9 Experiment 78; ninety-one days after the lesion. Right, no dorsal flexion of ankle and paw; weak extension of hip and knee; direct stimulation of nerves showed *E. P.* (right) all involved.



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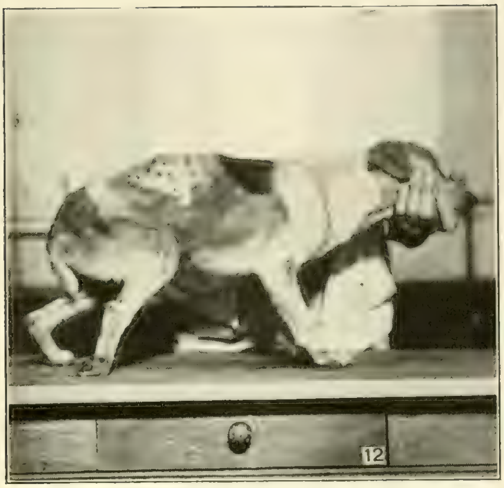
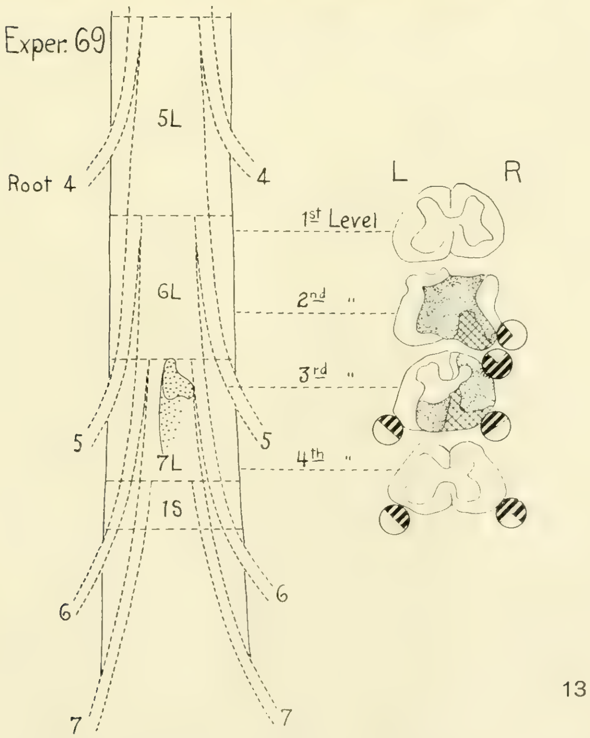
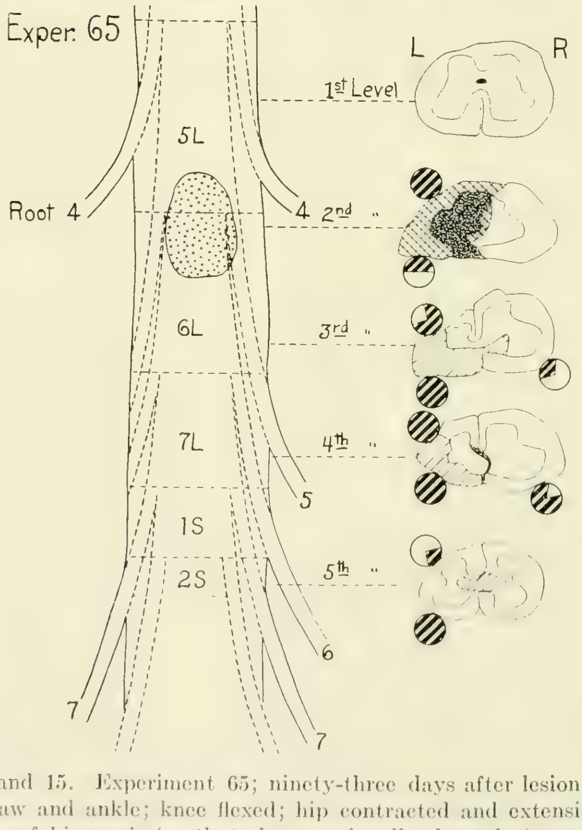
Figs. 10 and 11 Experiment 86; seventy-one days after lesion; condition little changed two to three months later, viz.: right, no dorsal flexion of paw and ankle; weak plantar flexion of toes; knee quite weak in extension; hip usually held flexed and also weak, left, stronger than right; dorsal flexion of paw and ankle gone; hip and knee slightly weak. Before killing, direct stimulation of nerves showed Right, *I. P.*, slight response in toes; no movement in ankle. *E. P.*, no response; left, *I. P.*, good response in toes; good response in ankle; *E. P.*, no response.





Figs. 12 and 13 Experiment 69; eighty-five days after lesion. Right, dorsal flexion of paw and ankle weak.



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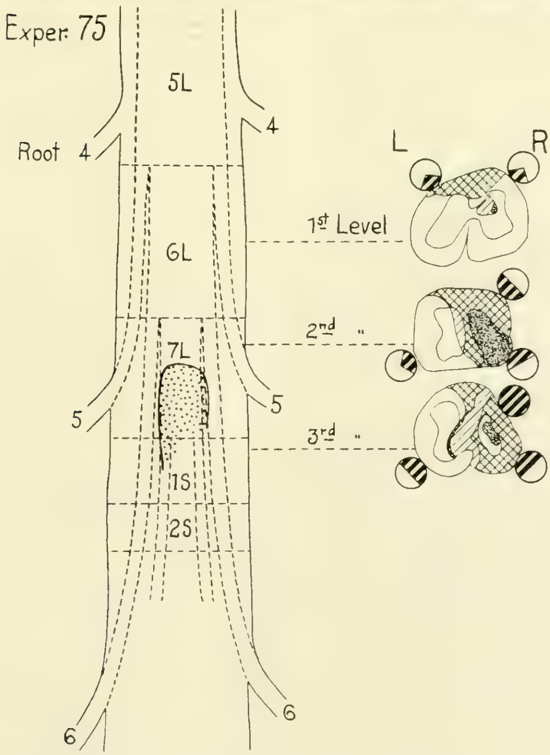
Figs. 14 and 15. Experiment 65; ninety-three days after lesion. Left, bad control of paw and ankle; knee flexed; hip contracted and extension lessened. Right, flexion of hip weak (so that she occasionally drags leg); weak power in extension of knee.



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Figs. 16 and 17 Experiment 75; ninety-four days after lesion. Right, no control except in flexion of hip; leg held under her, being flexed and adducted at hip; knee and ankle held straight and stiff; paw probably paralyzed and is held flaccid; glutei and hamstrings weak; quadriceps partially gone but contracted.

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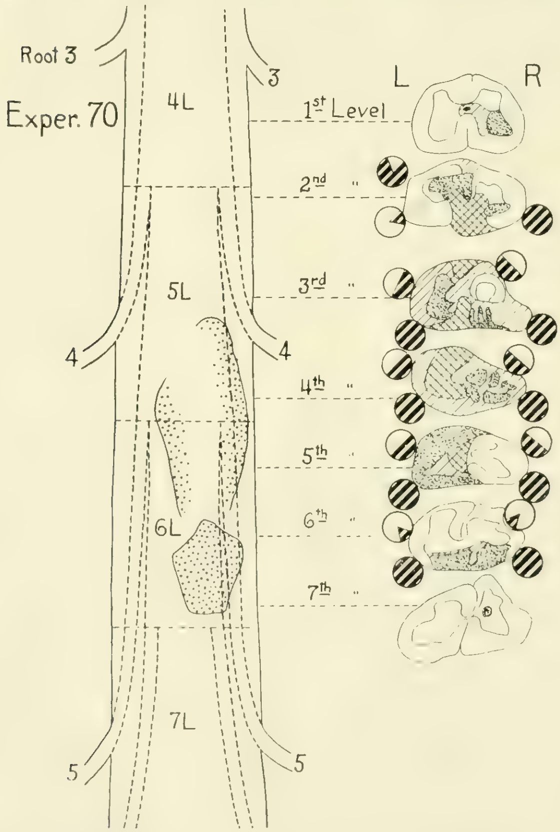

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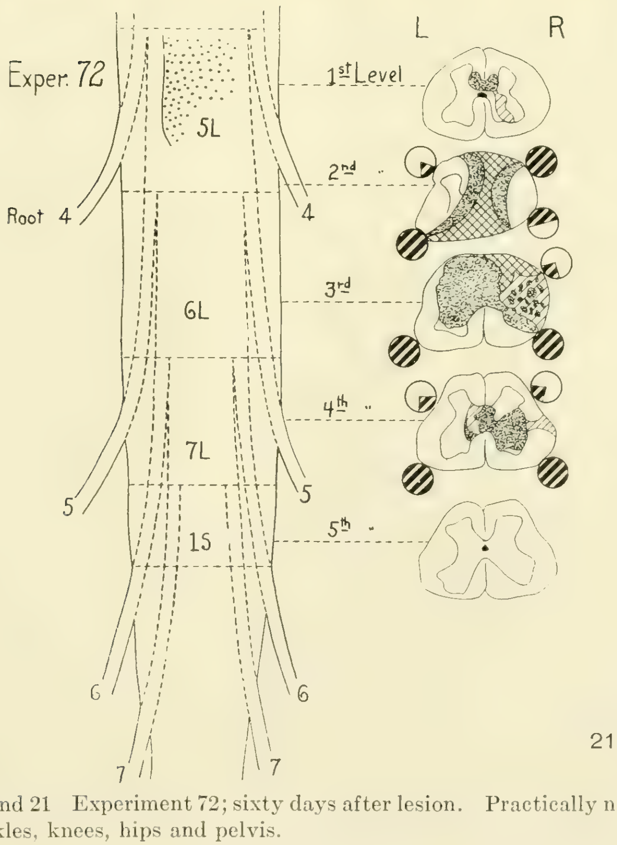
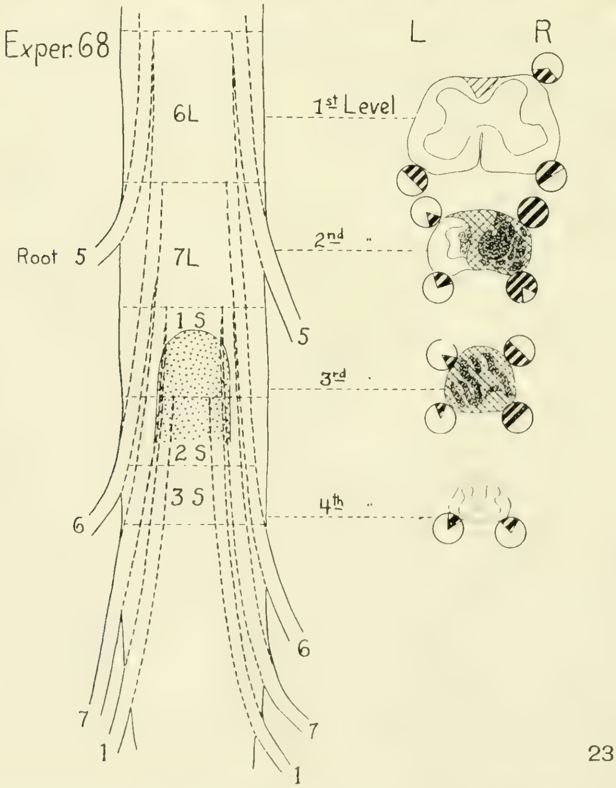
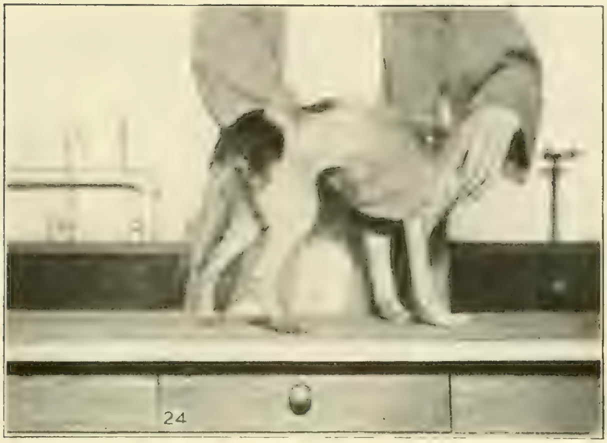
Figs. 18 and 19 Experiment 70; one hundred and twenty-three days after lesion. Poor control of pelvis, hips, knees, ankles and paws, but for the most part, not an atonic, flaccid paralysis. All joints are held permanently flexed to about normal angles, except the hips which are more extended than usual; spine bent convexly backward. On both sides erector spinae, glutei and quadriceps all weak; hamstrings fairly good; paws turn under occasionally; drags legs in position described.

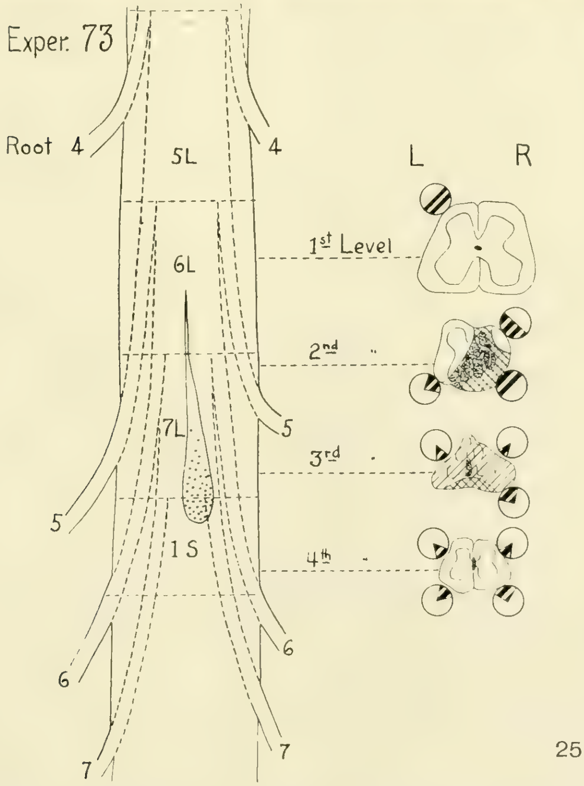


Figs. 20 and 21 Experiment 72; sixty days after lesion. Practically no control of paws, ankles, knees, hips and pelvis.



Figs. 22 and 23 Experiment 68; sixty-two days after lesion. Tail, mostly paralyzed; some power in root. Right, dorsal flexion of paw weak.





Figs. 24 and 25 Experiment 73; sixty days after lesion. Tail, mostly paralyzed; moves root a little.

Fig. 26 Experiment 68; one hundred and thirty-nine days after lesion; fifty-four days after fusion. Practically no control of tail.

Fig. 27 Experiment 68; two hundred and nine days after lesion; one hundred and twenty-four days after fusion; showing extent of control of tail in lifting at this date.

THE INFLUENCE OF AGE, SEX, WEIGHT AND RELATIONSHIP UPON THE NUMBER OF MEDULLATED NERVE FIBERS AND ON THE SIZE OF THE LARGEST FIBERS IN THE VENTRAL ROOT OF THE SECOND CERVICAL NERVE OF THE ALBINO RAT

ELIZABETH HOPKINS DUNN

From the Hull Laboratory of Anatomy, The University of Chicago

SIX FIGURES

So much has been published regarding the growth of the medullated nerve fiber that an explanatory word may be permissible on offering a paper which duplicates in many particulars the findings of other investigators.

The collection of the data presented here was suggested in 1903 by an examination of the control, or normal, material used by Dr. S. W. Ranson for his study of the spinal ganglion. Of the sectioned material, that for five rats of the thirty-one here reported was generously furnished by Dr. Ranson. In examining that material the salient feature was, to me, the increase in size of the medullated nerve fibers in the older rats, and it seemed desirable to ascertain if possible what conditions other than age might influence the size of these fibers. This point seems to have attracted other interest, since in 1906 Dr. Boughton published important data regarding the increase in size with age and weight of the medullated nerve fibers of the oculo-motor nerve in the albino rat and in the cat from a mixed series of males and females.

While the chief point of my inquiry was so admirably elucidated by Dr. Boughton's paper, there seemed to be place for the more extended study with defined categories which I had undertaken. Inquiry was made before continuing the work as to the existence

of any further duplicating studies. When it was learned later that Mrs. M. H. S. Hayes had a considerable series of counts of the ventral root nerve fibers of the second cervical nerve for the albino rat, postponement of publication was made for more than two years, but as her material has not been published it has seemed expedient to present my own findings with all due apologies to others.

With age as the constant factor in each group it seemed wise to vary the other factors which might determine the size of the largest nerve fibers. The additional factors selected were sex, body size as shown by extremes of weight within the limits of health, and relationship as shown by conditions within the same litter or in different litters. To these ends seven groups of rats were selected ranging in age from seven days to two hundred and seventy days, or nine months. These two limits were determined upon, the first because technical difficulties made accuracy in measurement among younger rats rather uncertain and the second because it had been found difficult to maintain good health and normal weight among laboratory rats after nine months of life. Each one of these seven groups was made up of four rats of the same age but of as widely varying weights as could be secured among healthy animals. The emphasis was laid upon the selection of light and heavy females, light and heavy males, thus including two females and two males in each group. Further information concerning conditions within and without the litter was secured by selecting certain groups from one litter and other groups from scattered individuals. The four rats of seven days, three of fourteen days and the one rat of one hundred and thirty-eight days are of one litter. The four rats of the group at thirty-six days are of one litter. The three rats of seventy-five days are of one litter. The four rats of the group at two hundred and seventy days are of one litter.

To this series of seven groups was added a group of aged rats, three males, of widely varying weights but not in good health. These rats were about six hundred and forty days of age.

As previously stated, the medullated nerve fibers of the ventral root of the second cervical nerve were selected for study and for

the purpose of uniformity the fixed point of section was opposite the spinal ganglion and central to the fusion of the two roots.

The methods of preparation of the material were those used for the enumerations and measurements in the leopard frog, (Dunn, '00, '02, '09) and differed but slightly from those of Boughton, ('06) Donaldson and Hoke ('05) and those of other investigators with whose findings comparison will be made. The nerve roots, ganglion and a portion of the nerve were fixed and stained unseparated in a one per cent solution of osmic acid, imbedded in paraffin, cut to a thickness of 4 micra and mounted serially.

The counts were made by the aid of an ocular net, and the measurements with an ocular micrometer, using an oil immersion lense at a magnification such that ten of the subdivisions of the ocular micrometer equalled one of the stage micrometer, or one one-hundredth of a millimeter. One division equalled 1 micron. This magnification facilitated greatly the final computations which were at best very tedious and required the closest control to eliminate the chances for error.

In selecting the nerve fibers for measurement, the entire section was surveyed and the largest fibers methodically selected. Each fiber was measured in two diameters, and its axis cylinder immediately measured in the same diameters.

The counting was done in daylight and the measurements taken under an oil immersion lense by the aid of an electric microscopic bulb of thirty-two candle power.

It was at first intended to select a variable number of nerve fibers for measurement, making the number proportional to the number of medullated nerve fibers in the section. But this plan was abandoned in favor of that of a fixed number which showed more accurately the increase in size at successive ages. The number fixed upon was ten. The results of this examination are found in tables 1 and 2.

Table 1 shows individual by individual, the age, the sex, the weight in grams, and the number of medullated nerve fibers in the ventral root of the second cervical nerve. Then for the ten largest medullated nerve fibers, the average diameter in micra, and the average area in square micra for the fibers, and the

TABLE 1

Records from measurements on the ten largest nerve fibers in the ventral roots of the second cervical nerve in a number of albino rats

Litter	Days	Sex	Weight	Number fibers	Ten largest fibers: Average diameter μ	Ten largest fibers: Average area □μ	Their axis-cylinders: Average diameter	Their axis-cylinders: Average area	Ratio of areas
			Grams						
A...	7	Female	8.25	368	4.60	16.61	3.60	10.18	1 : 1.63
A...	7	Female	8.93	368	4.75	17.80	3.75	11.00	1 : 1.62
A...	7	Male	8.90	372	5.05	19.95	4.05	12.82	1 : 1.56
A...	7	Male	9.75	360	5.60	24.63	4.55	15.07	1 : 1.63
A...	14	Female	19.98	518	7.05	38.93	4.85	18.40	1 : 2.06
B...	14	Female	21.85	566	6.95	38.04	4.75	17.79	1 : 2.14
A...	14	Male	20.73	536	6.60	34.13	4.50	15.90	1 : 2.21
A...	14	Male	21.93	594	6.35	31.59	4.30	14.52	1 : 2.19
C...	36	Female	39.17	651	9.35	68.81	5.80	26.42	1 : 2.38
C...	36	Female	45.30	654	10.55	87.58	6.75	35.89	1 : 2.44
C...	36	Male	31.20	536	10.36	84.30	6.50	33.18	1 : 2.51
C...	36	Male	52.65	689	9.90	76.98	6.20	30.19	1 : 2.55
D...	75	Female	130.31	505	12.15	115.75	7.90	49.01	1 : 2.36
D...	75	Female	143.10	615	12.10	114.99	8.00	50.26	1 : 2.29
D...	75	Male	149.40	609	12.15	116.13	8.20	52.81	1 : 2.20
E...	74	Male	189.70	726	12.25	117.67	8.20	52.81	1 : 2.23
F...	133	Female	161.00	634	12.70	126.68	8.25	53.58	1 : 2.18
G...	133	Female	167.51	731	13.60	145.27	9.10	65.03	1 : 2.23
A...	138	Male	260.00	680	13.16	133.96	8.84	61.37	1 : 2.18
H...	132	Male	274.00	569	13.70	148.04	9.10	65.03	1 : 2.27
I....	180	Female	200.00	510	14.55	166.50	9.80	75.43	1 : 2.20
J...	180	Female	225.00	526	14.75	171.11	9.85	76.36	1 : 2.24
K...	180	Male	227.60	546	15.45	187.23	10.65	88.91	1 : 2.11
L...	180	Male	302.00	662	16.55	215.38	11.70	107.51	1 : 2.00
M ..	270	Female	165.85	698	18.20	260.16	13.10	134.78	1 : 1.94
M ..	270	Female	187.96	853	18.25	261.87	12.95	131.92	1 : 1.98
M ..	270	Male	330.68	576	16.25	207.65	11.30	100.28	1 : 2.06
M ..	270	Male	349.41	658	16.95	225.91	12.05	113.85	1 : 1.98
N[1] ..	Aged	Male	210.00	901	14.85	173.43	10.50	86.59	1 : 2.00
O[1] ..	Aged	Male	379.40	934	15.00	176.69	10.00	78.54	1 : 2.25
P[1] ..	Aged	Male	414.00	758	14.35	161.96	9.40	69.40	1 : 2.33

[1] These aged rats were about 640 days old. They were killed in 1903, 1904 and 1907.

average diameter and the average area for their axis cylinders; and, in the final column, the ratio of the area of the axis cylinder to the area of the entire fiber.

TABLE 2

Averages for the enumerations and measurements for the groups of albino rats specified in table 1

	WEIGHT	NUMBER FIBERS	AVERAGE AREA TEN LARGEST FIBERS	AVERAGE AREA THEIR AXES	RATIO OF AREAS
	Grams				
7 days					
Two females.....	8.59	368	17.21	10.59	1 : 1.62
Two males.......	9.33	366	22.29	13.94	1 : 1.60
Both..........	8.96	367	19.75	12.27	1 : 1.61
14 days					
Two females.....	20.92	542	38.48	18.10	1 : 2.13
Two males.......	21.33	565	32.86	15.21	1 : 2.16
Both..........	21.12	554	35.67	16.65	1 : 2.14
36 days					
Two females.....	42.24	653	78.19	31.16	1 : 2.51
Two males.......	41.93	613	80.64	31.69	1 : 2.54
Both..........	42.08	633	79.42	31.42	1 : 2.53
75 days					
Two females.....	136.70	560	115.37	49.63	1 : 2.32
Two males.......	169.55	668	116.90	52.81	1 : 2.21
Both..........	153.13	614	116.14	51.22	1 : 2.27
132 days					
Two females.....	164.26	683	135.98	59.31	1 : 2.29
Two males.......	267.00	625	141.00	63.20	1 : 2.23
Both...........	215.63	655	138.49	61.26	1 : 2.26
180 days					
Two females.....	212.50	518	168.83	75.89	1 : 2.22
Two males.......	264.80	609	201.30	98.21	1 : 2.02
Both..........	238.65	564	185.06	87.05	1 : 2.12
270 days					
Two females.....	176.91	776	261.02	133.35	1 : 1.96
Two males.......	340.05	617	216.78	107.07	1 : 2.02
Both...........	258.48	697	238.90	120.21	1 : 1.99
640 days					
3 males..........	334.47	864	170.69	78.18	1 : 2.19

Table 2 introduces the averages for the groups of rats, first according to sex, and finally as a whole. It shows the average weight, the average number of medullated nerve fibers, the average areas of the largest nerve fibers in square micra, the average areas of their axis cylinders, and the ratio of these averages.

It may be of interest to show how the individual rats of the selected series differ from such averages as have been established already for the weight-age complex. In a joint paper by Donaldson, Watson, and Dunn ('06) certain averages and extremes of weight by age were published, upon which I have drawn for a series comparable with the present series. These data are presented in table 3 and are followed in table 4 by a compilation of the ages and weights of the rats of the present study.

TABLE 3

DAYS	MALES			FEMALES		
	Lightest	Heaviest	Average	Lightest	Heaviest	Average
7	7.3	12.7	9.2	7.5	11.8	8.7
14	14.0	17.6	15.2	13.5	18.1	15.6
37	28.5	48.0	37.8	29.8	47.4	39.5
76	89.8	157.5	121.3	89.6	131.6	110.4
131	132.4	249.2	202.5	151.2	214.7	178.6
178	167.9	291.2	239.4	153.0	215.0	191.7
256	190.5	310.0	265.4			

TABLE 4

DAYS	MALES		FEMALES	
	Lightest	Heaviest	Lightest	Heaviest
7	8.90	9.75	8.25	8.93
14	20.73	21.93	19.98	21.85
36	31.20	52.65	39.17	45.30
75	149.40	189.70	130.31	143.10
132	260.00	274.00	161.00	167.51
180	227.60	302.00	200.00	225.00
270	330.68	349.41	165.85	187.96

A comparison of the records for the approximate ages shows that, while the individuals of the present inquiry are not the lightest or the heaviest which might be obtained, they vary in a satisfactory degree from the averages given for males and females of comparable ages. I am not able to state the relationships of the Watson groups of rats but in my own groups of the younger rats a number of litters was included for each age.

The young of any given litter tend at birth to be of like weights and, so far as the argument can be drawn from records of the members of one litter, such related rats if differing initially in weight tend to approach each other in weight as growth goes on, if the conditions of growth are favorable (Dunn, '08).

Donaldson ('08, p. 353) says, "It is a familiar fact that rats even of the same litter and reared together grow very differently and therefore at the same age may have widely different body weights." Unfortunately we have no published data to show the initial body weight for related rats which differ so greatly at maturity. It appears, however, that unrelated rats and those reared under different conditions exhibit less uniformity of growth. The present findings suggest that these variations from the average may make themselves apparent in the nervous system as well as in the body weight. This is shown in the group at one hundred and eighty days of unrelated rats which, while fitting into the scheme for body weight, give numbers of fibers which are less than might be expected.

In all the findings which refer to weight the females must be considered separately from the males, at least after sexual maturity, since the growth curves for body weight differ greatly. For the curves showing this, reference may be made to Donaldson's papers, chiefly the collaboration with Watson and Dunn ('06). The body weight for the male rat increases for a longer period than that for the female and the average body weight for the adult male is considerably greater than that for the adult female of the same age.

The findings for this series of albino rats group themselves under two heads, first, the factors influencing the number of medullated nerve fibers, and second, those influencing the size of the nerve

fibers. The records for the individual rats are to be found in table 1. For the group averages table 2 must be consulted. Of the figures fig. 1 gives in *C* a curve based upon the averages of the groups for the body weight-age comparison, and in curves *B* and *A* the comparison of age with the size of the largest fibers and of their axis cylinders. Fig. 2 gives two curves related directly to the number of medullated nerve fibers. Curve *D* has been plotted for the fiber-age complex, and Curve *E* for group-weight averages and number of fibers. Both curves of fig. 2 show considerable irregularity in the later stages of growth. There is shown, however, a tendency for the number of medullated nerve fibers to increase in relation to both age and weight. The curves for the two are quite different, since the increments of age are daily periods, while the body weight is laid on chiefly during early life, as is shown by Curve *C*, fig. 1, by which two-thirds of the maximum average body weight is found to be present at one hundred and eighty days of life.

THE NUMBER OF MEDULLATED NERVE FIBERS

It is a difficult matter to estimate the direct relation of the number of medullated nerve fibers in the ventral root of the second spinal nerve in the albino rat to the body weight if the attempt to do so is made to the exclusion of the age factor. Especially is this true in the present series in which the extremes of weight at definite ages have been selected. So the attention has been directed to the variations to be observed within the group of one age. This number-weight relation must be considered for the sexes separately. If this is done the statement may be made that of two female rats of the same age or two male rats of the same age the heavier rat tends to have the greater number of medullated nerve fibers. This is especially noticeable when the compared rats are of the same litter. Unrelated rats are more likely to vary from this rule. This finding in regard to weight corroborates that of Mrs. M. H. S. Hayes in her unfinished work quoted by Hatai ('08, p. 154) with the added statement that this rule applies to the sexes separately.

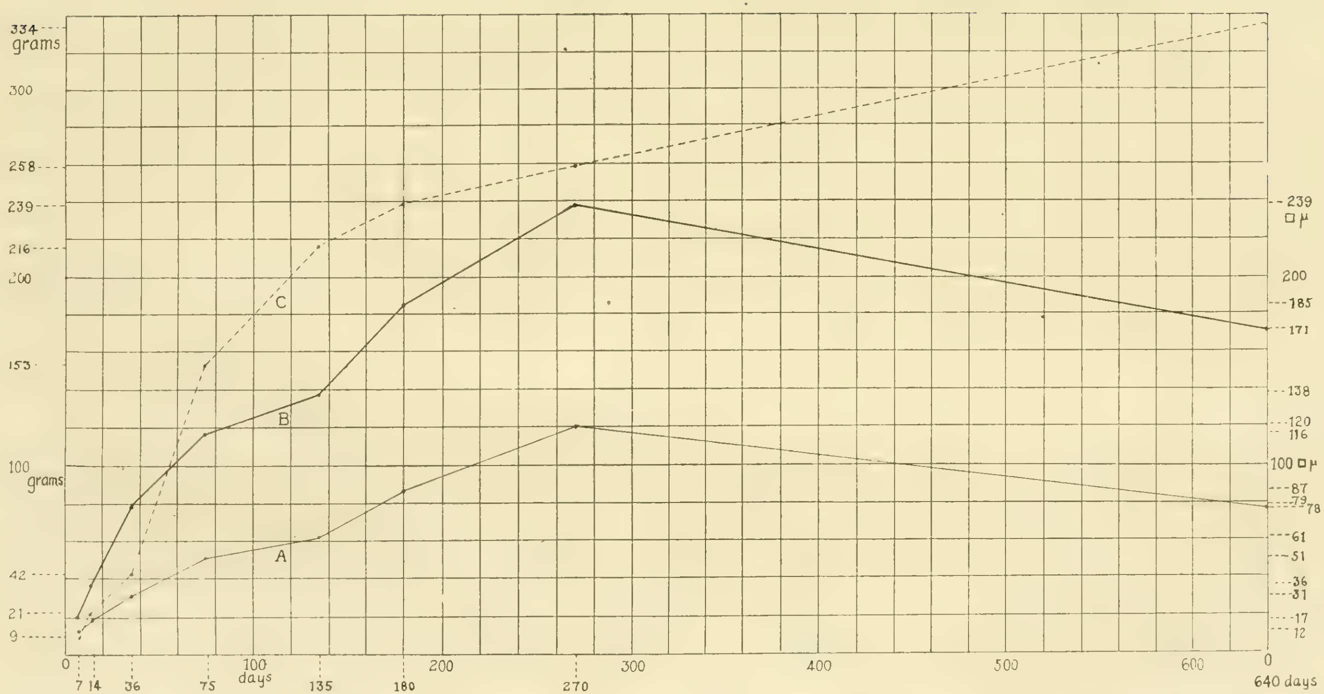


Fig. 1 *A* and *B* are plotted to show, for the groups of four rats at various ages, the average size of the largest fibers (*B*) and the average size of their axis cylinders (*A*), table 2. *C*, in broken lines, is the body growth curve for the rats from which the records for *A* and *B* were taken.

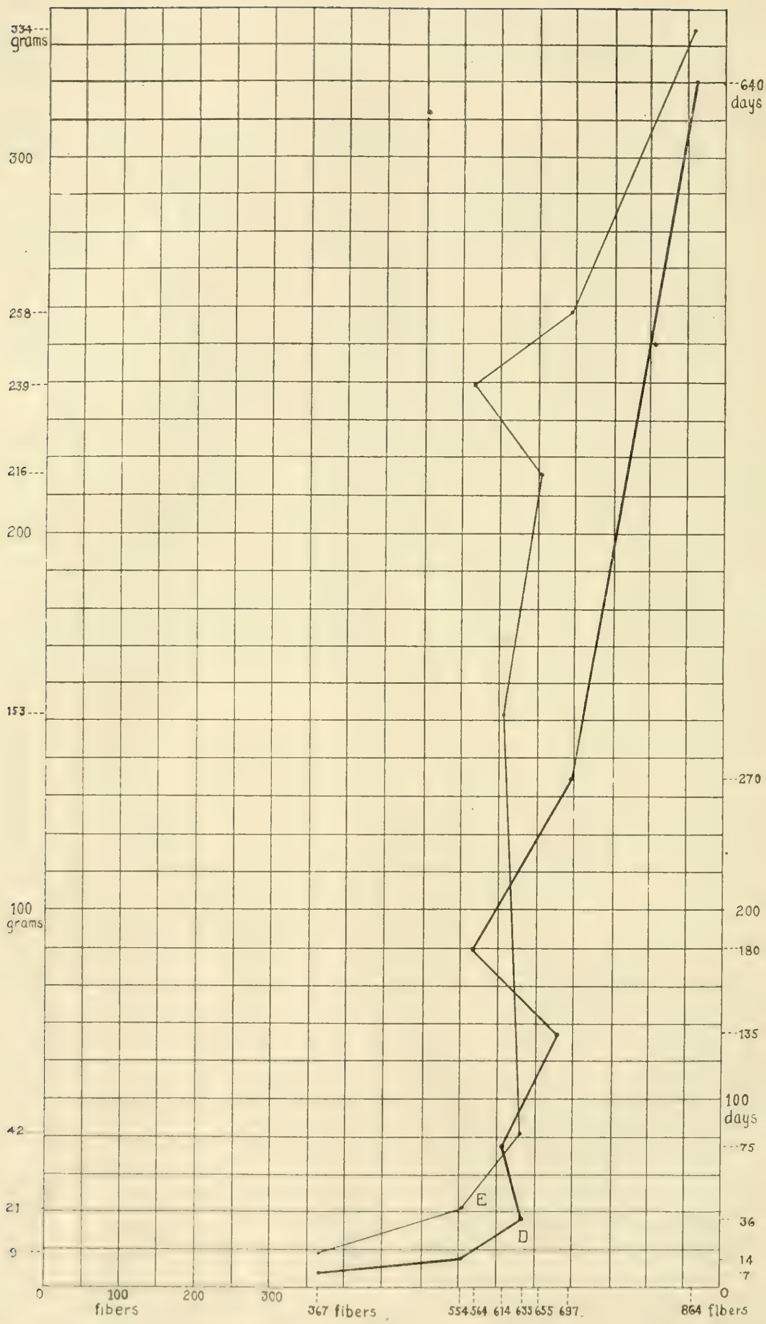


Fig. 2 *D* is plotted to show the averages of the number of fibers for the four rats of each group, at the ages studied. *E* is plotted to show the same averages of numbers in relation to the averages of body weight at the same ages.

Our findings seem to show that age has more influence on the number of medullated nerve fibers than has weight. In this regard our records disagree with the conclusion of Boughton ('06) for the oculomotor nerve of the albino rat. Reference to individual records in table 1 shows very definitely that there is no such relationship between body weight and the number of medullated nerve fibers that two rats of different ages but the same body weight will be more likely to have the same number of medullated nerve fibers in the ventral roots of the second spinal nerve than those of the same age but different body weights.

The statement also seems justifiable that rats of like age and of one litter tend to have the same number of medullated nerve fibers.

Curve *E*, fig. 2, has been plotted to show the relation of the averages for groups of rats of given ages to the averages for the number of their nerve fibers, but as this curve introduces the age factor in addition to that of weight, it will be discussed in the following section.

A considerable increase in the number of medullated nerve fibers occurs during the early life of the albino rat. This was noted by Hatai ('03) for the ventral roots of the sixth cervical, the fourth thoracic and the second lumbar spinal nerves, and by Boughton ('06) for the oculomotor cerebral nerve. In the second spinal nerve the increase seems to be checked at an earlier period than in the regions mentioned by Hatai. After the thirty-sixth day of our series, (table 2 and fig. 2) there is no uniform increase in the number of medullated nerve fibers, although the increments of age are considerable.

Hatai ('03) using the criterion of body weight, argued that the increase in number among the ventral root fibers continued to a later period. His maximum of weight was 264.3 grams, which might correspond to that of one of our one hundred and thirty-two day rats. Hatai's conclusions were based on the findings from one rat of each weight, age and sex not noted.

Boughton ('06) while noting age, weight and sex in his series for the oculomotor nerve, did not select his material systematically with respect to these factors. He obtained the maximum number of medullated nerve fibers in a male of one hundred and thir-

teen days and with a body weight of 278 grams. However, his curve immediately drops to a number not exceeding by one fiber the record for a male of seventy-seven days and 213 grams body weight. Tozer and Sherrington ('10) argue that the oculomotor is a mixed nerve.

Ranson ('08) made a few records for the number of ventral root medullated nerve fibers of the second cervical nerve in the albino rat in order to show the ratio of ventral to dorsal root nerve fibers. While his records for the dorsal roots begin with twelve day rats his counts for the ventral root fibers are only for seventy-two day and six month rats. The level of the count, the weight and sex of the rat are not stated.

His records for the dorsal root fibers give no stage between twelve and seventy-two days but show a marked increase in number at the later age. Of the ventral root fibers the average number of fibers for four seventy-two day rats is six hundred and thirty-two while that for two six month rats is seven hundred and thirty-six fibers. My own averages for seventy-five day rats and nine month rats give six hundred and fourteen fibers for seventy-five days against six hundred and ninety-seven fibers for the nine month rats.

Ranson's records then are comparable with those presented now and together they show that in regard to the second spinal nerve of the albino rat the number of medullated nerve fibers in both the dorsal and ventral nerve roots increases during the life of the individual but that the greatest increase occurs before the sexual maturity or so-called puberty of the animal.

More than this, if comparison between Ranson's records for the dorsal roots and my own records for the ventral roots of other individuals is permissible, the statement is possible that the increase in number is relatively greater and extends over a longer time among the dorsal root nerve fibers than among the ventral root nerve fibers. The functional significance of this finding is not so great as it would be if the counts for the dorsal root fibers had been made peripheral to the ganglion when the fibers directly innervating sensory elements would have been counted. As it is, the records are for processes central to the ganglion and ending

in the central nervous system. More than this they are upon individuals other than those from which the ventral root fiber records were made. Comparable records may yet be secured for the material upon which the present report is made. The plane of the section which was necessary to make measurements on the ventral roots peripheral to the ganglia reliable made similar measurements upon the dorsal roots unreliable. If some control for the measurements can be devised, the counts will also be made. Whatever may be the significance of the presence of the added fibers the determination of the time and manner of their appearance must be of importance.

The albino rat becomes sexually mature about the beginning of the third month of extra-uterine life, or about the seventieth day, but its increase in body weight may continue, to the end of the second year of life. According to the present records the average daily increase in weight between the ages of seven and fourteen days is 1.74 grams, and the average daily fiber increase is twenty-seven fibers. The average daily increase between fourteeen days and thirty-six days is 0.95 grams and 3.6 fibers. The increments of age and weight are too great to establish these averages as final, but they undoubtedly show the general growth relations.

The rate of increase in the number of medullated nerve fibers, in essential agreement with Hatai ('03) and Boughton ('06) diminishes with age. In our series the fourteen day period marks the greatest increase.

That such an increase in the number of medullated nerve fibers occurs in other animals has been shown by Boughton ('06) for the cat and suggested by Willems ('11) for the rabbit. Boughton ('06) used a mother cat and her five kittens, two males and three females. An increase in the number of medullated nerve fibers of the oculomotor nerve is shown among the kittens from one day to one hundred and eighty-two days, with the exception of the female at ten days, but the mother cat at thirteen years has a number of fibers not quite equal to that of the kitten of fifty-six days.

Willems' records ('11) were offered to show the relation of large and small nerve fibers in the portio minor of the fifth cerebral

nerve and its branches in various adult rabbits but, unfortunately for comparison with other records, do not state the age or sex of the animal. There is shown however in his table 4 (p. 143) a variation in the total number of medullated nerve fibers in the motor portion of the fifth cerebral nerve which might indicate the influence of an age factor.

It would seem from the present more extended series from albino rats that the increase in the number of medullated nerve fibers practically ceases with the attainment of the adult condition and that the presence of an individually greater number after that period is dependent upon some factor determining the number to be supplied to the adult body and is not likely to be due to the maturing of immature elements in the nervous system as it seems to be at the earlier ages.

This increase in number of medullated nerve fibers with the increase in weight and age may have several interpretations. When it appears as a condition most marked during the period of rapid growth, it may well seem to be associated with the increase of the number of body elements which must be innervated. During the same period, or more naturally later, it may be dependent upon an increased innervation of already innervated material.

Our data as regards the number of innervated peripheral elements either sensory or motor are extremely limited.

Intimately bound up with this question is that of the splitting of peripheral nerve fibers, and no absolute correlation between the number of nerve fibers in the spinal roots and the number of peripheral innervated elements can be made with our existing knowledge.

It would appear in general that the increase in size of the immature body is accomplished by the increase in both number and size of the individual elements, while that of the mature body is rather due to increase in size of the already formed elements.

Boughton's ('06) findings lead him to state that after the initial laying down of nerve fibers the added fibers never attain the size of the earlier formed fibers, and the added fibers must be small fibers. Under this interpretation size is determined by age and

these additional fibers whatever their function are determined by the time of their appearance to be small fibers.

This phase of the significance of the added fibers is so bound up with the discussion of size that we may consider it more readily under that topic.

SIZE OF THE MEDULLATED NERVE FIBERS

If the period preceding puberty marks the time of the chief increase in the number of medullated nerve fibers, it has no such notable relation to the increase in size of the medullated nerve fibers, which continues at least to the ninth month. This is clearly indicated in table 2, which gives the average areas in square micra for the ten largest fibers of four individuals in each group. In this table, and in fig. 1, curve *B*, an unbroken increase occurs for our averages from the seven day group to the two hundred and seventy day group. A somewhat similar increase occurs in the areas of the axis cylinders of the same fibers, showing that the integral portion of the nerve cell increases in size during health with increasing age at least to the ninth month of life.

The ratio of the average for the axis cylinder and for the entire fiber (table 2) gives some interesting information as to the growth of the medullary sheath. Using the average area of the axis cylinder as the unit, at seven days the area of the entire fiber is about one and one-half times that of the axis cylinder, that is the medullary sheath has half the area of the axis cylinder. At the succeeding stages of body growth to the two hundred and seventy day period the medullary sheath is thicker than the axis cylinder. The greatest relative thickness of the medullary sheath appears in our series at the thirty-sixth day when, contrasted with the unit area of the axis cylinder, the area of the medullary sheath is 1.47. From this age there appears a readjustment in the growth of the two, until at nine months the ratio is 1:0.99 or the one to one relation of the normal adult (Donaldson and Hoke, '05).

Among the members of our group of aged and infirm male rats the nerve fiber is affected in both axis cylinder and medullary sheath. The average area of the entire fiber and of the axis cylin-

der is considerably decreased. The axis cylinder is the more affected so that the ratio approximates that of a fourteen day rat or a hundred and eighty day rat, in which the medullary sheath has a slightly greater area than has the axis cylinder.

The curves for the averages have been plotted in fig. 1, Curves *A* and *B*. At seven days Curve *A* begins at 12 square micra and Curve *B* at 17 sq re micra. As age increases the curves diverge until at two hundred and seventy days the fiber is twice the size of the axis cylinder. In old age, six hundred and forty days, the axis cylinder has an average area of 78 square micra, while that of the entire fiber is 171 square micra, showing a distinct shrinkage in both axis cylinder and medullary sheath.

The comparison of the curves for the nerve fiber and for its axis cylinder with the curve for the body weight of the same animals is full of interest. Curve *C* has been plotted in the same figure to show the relation of the body weights of the groups to their age.

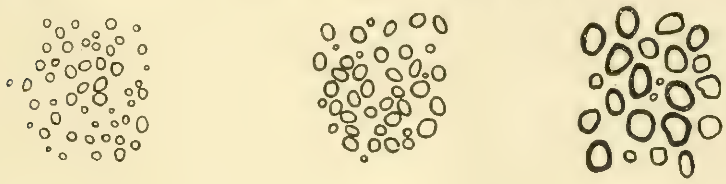
From this comparison we see that the size of the largest nerve fibers increases more rapidly than the weight of the body until the thirty-sixth day. From the thirty-sixth to the seventy-fifth day or until puberty the body growth is very rapid, overshadowing that of the nerve fiber. This disporportional growth of the body continues until about the one hundred and thirty-fifth day. At that age the body has acquired two-thirds of its ultimate size, while the largest nerve fibers have acquired only a little more than one-half of their ultimate size. The growth of the nerve fibers from the one hundred and thirty-fifth day is more rapid than the body growth, so that the curves approach one another at the two hundred and seventieth day. At six hundred and forty days, or old age, the curves are widely separated, due both to the increased weight of the body and to the decreased size of the nerve fibers.

It may be well to discuss more fully the question of size as related to all the nerve fibers.

Measurements have been made upon the largest nerve fibers for convenience and for accuracy. To those who have made the attempt, the difficulty of making such measurements with the ocular micrometer need not be emphasized. The difficulty of this

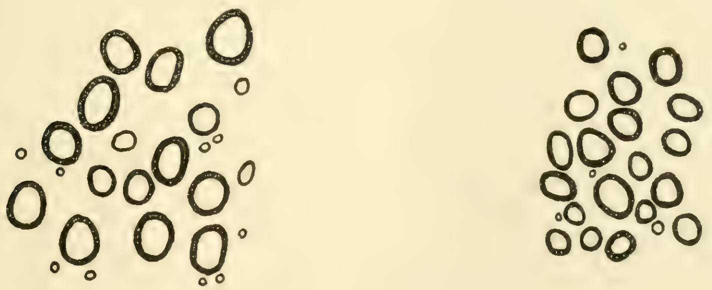
series was considerably increased by the handling of very young material.

These measurements prove the increase in the size of the largest medullated nerve fibers, but that the increase is not confined to the largest fibers is evident to one making even a casual study of



A B C

Fig. 3 Camera lucida drawings by A. B. Stredain at table level, ocular 8, objective 8, Zeiss, showing typical areas from the ventral roots of the second cervical nerve of three male rats of one litter. *A* at seven days, body weight 8.9 grams; *B* at fourteen days, body weight 21.93 grams; *C* at one hundred and thirty-eight days, body weight 260 grams.



D E

Fig. 4 Camera lucida drawings, at same magnification as those of fig. 3, showing typical areas from the ventral root of the second cervical nerve of two male rats. *D* at 270 days, body weight 349 grams; *E* at 640 days, body weight 414 grams.

the material. Fig. 3 was drawn by camera lucida to include typical areas from the ventral roots of the second cervical nerves of three male rats from one litter. *A* at seven days, *B* at fourteen days and *C* at one hundred and thirty-eight days. Fig. 4 gives drawings at the same magnification from two additional male rats, *D* at two hundred and seventy days, and *E* at six hundred and

forty days. The variation in size is too obvious to need further comment. *E* shows the decrease in size among the old rats. The increase in size is apparent among the majority of the fibers and may include all the fibers. Nevertheless some of the medullated nerve fibers may appear early and not continue their growth over the usual time or may grow more slowly. Small fibers may be destined to be small fibers from their first appearance.

Boughton ('06) made an attempt to classify the medullated fibers of the oculomotor nerve into 'large' fibers and 'small' fibers. One is somewhat puzzed regarding his method. According to his table 1, Boughton entered all the fibers present at eleven days as 'large' fibers, noting the addition of 'small' fibers at later ages. In his discussion of method (p. 156) he states that all his sections were photographed at the same magnification and only those fibers distinctly recognizable at that magnification were entered as 'large' fibers. If the material was at all comparable to that of the present investigation a magnification which would show all the fibers at the youngest period would show all the fibers at all the other periods. Our fig. 3 proves this.

The oculomotor nerve carries a considerable number of small medullated nerve fibers as viscero-motor fibers to the ciliary ganglion (Apolant, '96, p. 664). The presence of such fibers may account for the large number of small fibers found by Boughton ('06) in the oculomotor nerve of the albino rat. In such a group of nerve fibers, which plainly differs functionally from the somatic fibers, a distinct difference in size would be difficult to interpret alone from the standpoint of age of the fibers. All viscero-motor medullated nerve fibers have been recognized as small fibers. This was early stated by Gaskell ('86) and Langley ('96) and corroborated by many more recent investigators. It is possible that these fibers are later in medullation than those passing to the body muscles, but they may not grow so rapidly as do the somatic motor fibers.

Further, if we have in the third nerve which has been considered a purely motor nerve, a mixed nerve as Tozer and Sherrington ('10) have argued, the interpretation of Boughton's findings has an added complication as both efferent and afferent fibers would

be included. Such an interpretation has been given to the function of the ventral roots by Kidd ('11) and others. I have unpublished a note on the presence of medullated nerve fibers in the ventral roots of the spinal nerves in the leopard frog, which from the direction of their degeneration seem to have their cells of origin in the dorsal root ganglia. It may be then that in no nerve or spinal root are we dealing with unmixed fibers. The relative number of efferent fibers seems greater in the second cervical nerve than in any of those previously considered. Roth ('05) states as an argument in proof of the visceral character of the eleventh cerebral nerve that in the rat the ventral roots above the sixth cervical have a small number of small medullated nerve fibers, and that the second cervical nerve has in its rami communicantes few or no small medullated nerve fibers.

Willems ('11) has discussed the question of the size of medullated nerve fibers quite fully giving prominence to three theories and introducing one of his own based on the differences in size found among the branches distributed to various muscles from the efferent portion of the fifth cerebral nerve in the rabbit. Quoting from page 203, "Nous pensons donc que l'individualité des nerfs a son origine principalement dans la différente valeur de l'accroissement secondaire pour chaque muscle."

It has seemed to the writer that the propounders of these so-called theories as set forth by Willems have not attempted to put forth an all embracing theory, but have each one been attempting to define factors which may influence the size of the medullated nerve fiber. Each one has noted conditions which have run parallel with size and have been piling up evidence bit by bit. At the present moment it seems most probable that the size of the medullated nerve fibers must be interpretated finally as due to a combination of causes, and I anticipate that all the bits of information will be wrought into a complex mosaic when the final illuminating word can be said. One can readily see that in regard to size the neurones may appear in successive crops so that the earlier crop may have a greater size, according to Boughton's and Donaldson's attractive theory, while at the same time these successive crops have definite functional values which in turn may be influ-

enced by the richness of the fiber branching, by the size of the innervated elements, by the frequency of their use, or possibly, if neurofibrils are the conducting elements, by the number or size of the neurofibrils. However this may be, we must be grateful for every attempt to delimit the problem, while admitting that much further information is desirable.

Increase in size is indeed a matter of growth, and it is extremely confusing to the problem that age plays so large a part in determining the actual and possibly the relative size of the medullated nerve fibers. It would appear that the next step in the interpretation involves the accumulation of a mass of data regarding the direct relation of the size of the nerve fibers to the tissues innervated and much further information as to the relation of these successively appearing fibers to the peripheral structures. The latter involves a study of the axonic relations to the periphery and the time of medullation of these axones.

My own findings (Dunn, '02 and '09) on the distribution of the medullated nerve fibers to the segments of the leg of the leopard frog suggest that difference in time of outgrowth from the central nervous system is not sufficient to account for the appearance of the largest medullated nerve fibers in each instance in the segment nearest to the body. A peripheral factor seems to be present here.

At the moment it is sufficient to inquire as to the information regarding the factors determining the size of the medullated nerve fibers which may be drawn from the present investigation.

It has been pointed out that, unlike the oculomotor nerve, the number of medullated viscero-motor fibers is small, and that the number of very small medullated nerve fibers is much less in the adult than in a rat of seven days.

Among the possible influences upon the size of the medullated nerve fibers has been mentioned the size of the animal. Dhéré ('03) has shown that the extraction of myelin is greater among mammals of greater size than among those of less size. It will be interesting to inquire as to the influence of weight among the individual groups of white rats. Comparing females with females and males with males, table 1, in each group of fixed age, the

heavier individual tends to have the larger average size of the largest nerve fibers. To this law there are a few exceptions, but in each instance the individual of greater weight but with the smaller nerve fibers was found to have a greater number of nerve fibers in this spinal root.

The correlation, then, is not between body weight and size of the nerve fibers, but between body weight on the one hand and number and size of the nerve fibers on the other hand. Of the two factors, the size of the fiber is more affected by the body weight than by the number of fibers. The larger fibers may be found with greater body weight and greater number but when less size of fibers is found with greater body weight the compensating factor is the increased number of nerve fibers. This relation appears to be an argument in the establishment of a definite relation between the amounts of innervated and innervating material.

Size of the fibers appears to have little relation to the body weight when the sexes are considered together. There are but slight deviations in size of the largest medullated nerve fibers in males and females of the same age but of widely diverse weights.

Body weight without regard to age is misleading when used for a criterion of the size of nerve fibers. Selecting at random from table 1 a male and a female of a weight less than 200 grams, one might chance upon a female of 187.96 grams weight and a male of 189.70 grams weight. The male however is seventy-four days old, the female two hundred and seventy days of age. The male has seven hundred and twenty-six medullated nerve fibers in the ventral root of the second cervical nerve, the female eight hundred and fifty-three fibers. The average area of the ten largest medullated nerve fibers in the male is 117.67 square micra and of their axes 52.81 square micra, while for the female the average area for the fibers is 261.87 square micra and for their axes 131.92 square micra. This may be analogous to the condition found in the spinal cord by Donaldson ('08, p. 371). In those findings, in rats of the same body weight without regard to age, female rats were found to have heavier spinal cords than the males. The

amount of medullation and size of the fibers might increase the weight of the cord.

A further step in the comparison of males and females of the same ages but of different weights is the recognition of the fact that among mature rats, the females, which rarely attain the weight of the males, must have a mass or bulk of the peripheral nervous system much greater in proportion to the body weight

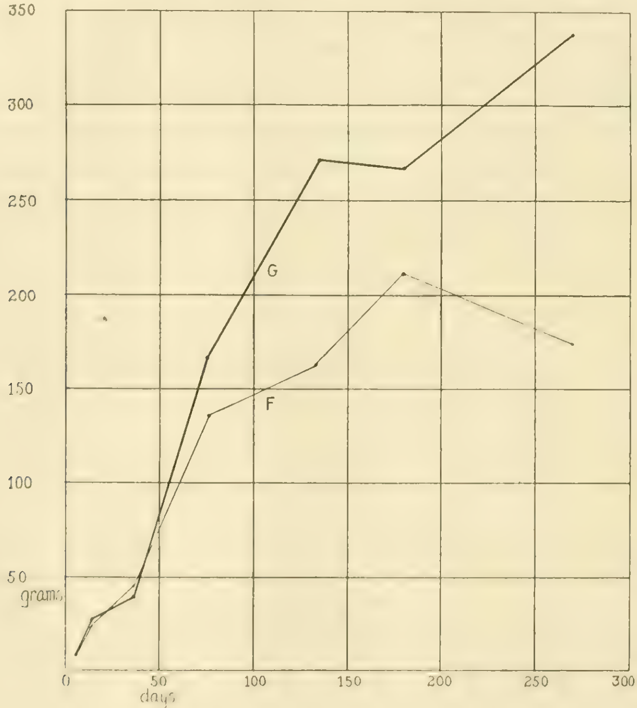


Fig. 5 *F* is plotted to show the body growth for the female rats of each group and *G* the curve for the male rats of the same groups. These curves are to be contrasted with those in fig. 6, which show the curves for the growth of the largest nerve fibers.

than that of the males. Increased weight in the spinal cord in the female has been interpreted as due to the richness of visceral innervation, especially of the pelvic organs. But in this second cervical nerve an excess over the males is found with very few or no

viscero-motor medullated nerve fibers, so that the proportional greater pathway for impulses is furnished by the somatic motor system. An interpretation of this condition is difficult, since there seems to be nothing in the bodily functions of the muscles to require or explain richer somatic innervation in the females of a given age. In plotting the curve for the number of rats used in this investigation there seems to be no association between the

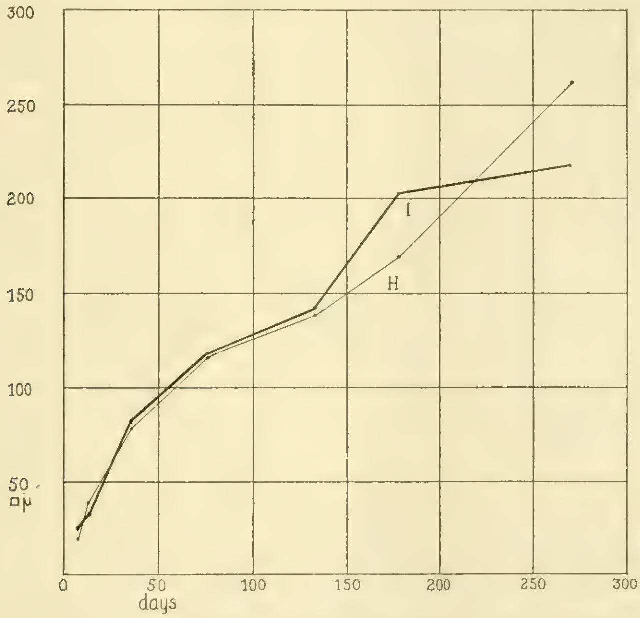


Fig. 6 *H* is plotted to show the growth curve of the largest nerve fibers for the female rats of each group, and *I* for the male rats of each group (table 2).

early checking of body growth in the female and the growth of the nerve fiber. The latter goes on as rapidly or more rapidly in the female after the checking in rapidity of body growth as it does in the male with a more prolonged growth. A comparison of fig. 6 with fig. 5 makes this clear. Fig. 5 gives the curve for body growth for the two males (*G*) and the two females (*F*) of each group. Fig. 6 gives the curves for the nerve fiber growth of the same rats, *H* for the females and *I* for the males.

In the interpretation of all investigations on the albino rat much gratitude is due to Professor H. H. Donaldson now of The Wistar Institute of Anatomy and Biology at Philadelphia, for his preliminary studies upon the rat. Growth questions in general are of especial value to us with reference to human young. Professor Donaldson over a long period of years, more than ten to my knowledge, has been inspiring a series of studies of a more and more critical nature upon the albino rat as preliminary and comparative studies to those on the human body. An outline of some of his plans accomplished and projected is to be found in the President's address before the Philadelphia Neurological Society (Donaldson, '11).

While the present investigation was undertaken independently, it owes much for its interpretation to the solid and illuminating researches just mentioned. It is interesting to find a laboratory animal which in so short a lifetime as three years so conveniently reproduces many of the conditions found in the human being. because of this recapitulation, growth conditions in the peripheral nervous system of the albino rat have the added value of aiding in the interpretation of anatomical and functional conditions in the human body.

CONCLUSIONS

During the period of rapid growth, shown in the albino rat in the accompanying tables from the seventh to the thirty-sixth day, there is a parallel increase of the number of medullated nerve fibers in the ventral root of the second cervical nerve. When the sexes are considered separately, the heavier individual at each age has the greater number of medullated nerve fibers. Neither of these relations is so definitely marked among the adult rats.

In this series of observations there is found a continuous increase in the size of both the medullated nerve fibers and their axis cylinders to nine months of age. Among the old rats about six hundred and forty days of age, there is a noticeable decrease in size from that of the nine month rat both in the nerve fiber and its axis cylinder.

The growth of the medullary sheath as compared with that of the axis cylinder is emphasized in this series. At seven days the area of a cross section of the axis cylinder is noticeably larger than that of the medullary sheath. After that age the medullary sheath grows more rapidly than the axis cylinder, at fourteen days having a greater area than the axis cylinder. At thirty-six days it has one and one-fourth times the area of the axis cylinder. From thirty-six days the axis cylinder grows more rapidly until at nine months there exists the one-to-one relation of the adult which has been found in many vertebrates including the albino rat. In old age there is a relatively greater loss in the axis than in the medullary sheath, so that the area of the medullary sheath is the greater.

While male and female rats may be grouped together in the study of the influence of age upon the size of the medullated nerve fibers, accuracy demands their separation when the influence of weight is to be considered. The growth curve for the two sexes appears to be different for the individual elements of the nervous system, as it has been shown to be for the central nervous system and for the body.

Usually for both males and females of a fixed age the greater average area of the largest medullated nerve fibers of the ventral root fibers of the second cervical nerve is found with the greatest body weight. But if the less fiber area is found with greater body weight the number of fibers is always greater. Greater number may be found with greater area and greater body weight. This correlation between the body weight and the size of the medullated nerve fibers is an argument in favor of the theory of a certain relation between the amount of tissue to be innervated and the caliber of the innervating pathway.

In the later periods of growth among both males and females the size of the medullated nerve fiber runs more closely parallel with the body weight than among the more immature individuals, that is, growth processes in the individual nerve elements are more rapid and also more variable among the immature than among more mature individuals.

The comparison of mature males with mature females shows that, while the body weight of the females may be much less than that of the males of the same age, there is no marked difference in the size of the largest nerve fibers, so one may say in general that the largest nerve fibers in mature females are much larger in proportion to the body weight than are those of mature males of the same age, that is, the efferent pathway is greater in the female than in the adult male of the same age.

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THE STRUCTURE OF THE SPINAL GANGLIA AND OF THE SPINAL NERVES

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FIFTEEN FIGURES

The application to the spinal ganglion of the reduced silver method of Cajal has brought to light many new facts (Cajal, '05) and the renewed interest in this subject has found expression in a number of investigations including that of Dogiel ('08). The present paper is concerned, in part, with a confirmation of these newer observations and in part with observations which, though touched upon by Dogiel and Cajal, escaped serious consideration by either of them.

For this work the largest spinal ganglia (L VI, VII, S I) in large dogs were subjected to the pyridine-silver (modified (Cajal) technique, an account of which has already been published (Ranson, '11). Pieces of fresh nerve are placed for two days in absolute alcohol containing 1 per cent of concentrated ammonia; washed one to three minutes in distilled water; placed in pyridine for twenty-four hours, after which they are washed in many changes of distilled water for twenty-four hours. They are then placed in the dark for three days in a 2 per cent aqueous solution of silver nitrate at 35° C.; then rinsed in distilled water and placed for one day in a 4 per cent solution of pyrogallic acid in 5 per cent formalin. Sections are cut in paraffin and after mounting are ready for examination. With fresh pure chemicals, absolutely clean utensils, and a reasonably constant temperature this method can be relied upon to give uniform results.

[1] The work upon which this paper is based was done in the Anatomische Anstalt, Freiburg i. Br. My thanks are due to Professors Wiedersheim and Keibel, through whose courtesy it was possible for me to carry on the investigation.

The ganglia were cut into sections 18μ thick. It is difficult to make use of thicker sections because the silver method, unlike the intra-vitam use of methylene blue, brings out all the nerve elements so that very thick sections are confusing. On the other hand it is difficult except in thick sections to follow the axons to their bifurcation.

Types of spinal ganglion cells according to external form

1. The simple unipolar cell. From the body of a cell of this type arises a single axon, which after a longer or shorter, straight or convoluted course divides dichotomously into a thin fiber directed toward the spinal cord and a much thicker fiber running into the nerve. These represent, according to my observations, the great majority of the cells in the spinal ganglia of the dog. In sections 18μ thick it is only in a small proportion of the axons that the entire course from cell body to bifurcation can be seen. For the most part it is possible to follow the axons for only a limited distance before they leave the level of the section. Many axons can be seen dividing but these can rarely be followed back to their cells of origin. Nevertheless this connection is so well established that it needs no confirmation.

In the case of (a) the large cells, the axon is usually much convoluted in the immediate vicinity of the cell and within its connective tissue capsule, but in some cases the coil is wanting. In other cases the axon is very short and divides almost immediately after leaving the cell. These axons acquire a myelin sheath and stain by the pyridine silver method a light yellow.

(b) All of the small cells and some of the medium sized ones present a different picture (fig. 1). The axon (whose thickness varies with the diameter of the cell) is thin and stains a dark brown or black. These axons seldom make complicated coils about the cells, but run more or less directly toward the central fiber bundles of the ganglia. Here these fibers can be seen in large numbers dividing in the manner of a *T* or *Y* into a very fine centrally directed fiber and a somewhat thicker one running toward the nerve (fig. 2). These branches together with others

of similar origin and appearance form bundles of fine black fibers which can be followed into the dorsal root on the one side and into the peripheral nerve on the other. These axons are devoid of a myelin sheath (or according to Dogiel, some of them may present a thin and interrupted coat of myelin similar to that which is seen on some sympathetic fibers).

The axons in fig. 1 show about the average amount of coiling; but while in many cases they are almost straight, in a few the winding is as pronounced as that of the axon of any large cell. It is of interest to compare the bifurcation of these fibers with that of the medullated axons (fig. 2). These latter branch at a node of Ranvier and show a marked constriction both of the main stem and the two branches at the point of bifurcation. In none of the dividing non-medullated fibers is such a constriction to be seen. Instead there is a broad triangular area at the point of the bifurcation. These fibers differ then from the medullated in their small size, in the intensity with which they stain with silver, in the absence of a surrounding unstained ring of myelin, and in the manner of their bifurcation.

The description which is here given of the small cells and their axons is in no sense new. Dogiel in 1897 gave a very satisfactory account of them, which was confirmed by Cajal ('07) and later again by Dogiel ('08, p. 33). Dogiel found on some of these fibers very fine myelin sheaths which disappeared and reappeared from stretch to stretch along the fiber. It is strange that neither author gave any further consideration to these axons, apparently overlooking the fact that they outnumber the medullated fibers by as much as the small cells outnumber the large. We will return to the significance of these observations in another part of this paper.

2. Cells whose axons have collaterals ending in end bulbs. Dogiel's type II, Cajal's type VI. The peculiarity of these cells lies in the fact that the axon gives off fine collaterals, which after a course usually of no great length, end in characteristic swellings, which according to their location may be divided into three subgroups: (a) In the majority of the cases the collaterals are given off from the axon before it has left the connective tissue capsule

which surrounds its cell of origin. The collateral is usually short and directed toward the surface of the cell but may be long and coiled in its course. It ends in a bulb which lies upon the surface of the cell from the axon of which it arose (fig. 3). The terminal swelling may be very large in proportion to the size of the collateral, and in some cases the latter increases in thickness as it approaches the end-bulbs. These bulbs take only a light stain with silver, appearing bright yellow. They lie upon the surface of the cell beneath the connective tissue capsule and produce as a rule a depression of the cell surface. (b) Sometimes the collateral is given off from the axon at some distance from its cell of origin—and piercing the connective tissue capsule of another cell terminates in an end bulb which lies upon the surface of this second cell (fig. 4). (c) Still other collaterals run in the connective tissue of the ganglion and end there in bulbs surrounded by a special capsule. Sometimes two or three such bulbs lie together in a felt-work of fine fibers and the whole mass is surrounded by a capsule.

Huber ('96) was the first to describe these structures but saw only those that fall under subhead (a). Both Cajal and v. Lenhossék considered that some of these fibers were fine dendritic branches arising directly from the cell body. On this point my observations agree with those of Dogiel for in every case where the origin could be determined they arose as collaterals from axons and never as fine dendrites. According to Cajal and Dogiel the axons of cells of this group, after having given off the collaterals just described, end by dividing after the manner of a *T* or *Y* into central and peripheral fibers.

3. The axon of a cell of this type splits up into a number of branches which with or without further branching are finally reassembled into a single axon. In sections 18μ thick it is not possible to see in their entirety such complicated structures of this sort as were seen by Dogiel in his thicker sections and whole mounts; but the simpler forms are often included within a single section. A relatively common arrangement is seen in fig. 5. The axon divides into two fibers which may or may not be of equal size and which soon reunite. A somewhat more compli-

cated form is shown in fig. 6. Here the axon breaks up into a number of fibers which unite with each other to form a plexus out of which a single axon is again formed. Sections through much more complicated networks formed by splitting axons can often be seen.

4. In another variety, closely related to those just described, the axon arises from the cell by two or more roots, each of which has the appearance of an axon and forms a conical expansion at the point of origin from the cell. Each of these roots may branch repeatedly. These branches then reunite with each other forming a more or less complicated network, out of which a single axon arises (fig. 7).

Groups 3 and 4 are closely related in that the cells of the latter differ from those of the former only in the fact that the splitting involves the initial portion of the axon. In both cases it is rare to find a myelin sheath on the fine fibers forming the plexus. These two groups correspond to Dogiel's types v and vi taken collectively, but the basis of separation into the two groups is different. It is quite bewildering to read Dogiel's description of types v and vi with their subvarieties and try to determine what the basis of classification of his seven subvarieties into these two major groups might have been. For this reason it has seemed best to adopt as a basis of classification the more obvious and apparently more fundamental difference in the origin of the axon by a single or by a number of roots.

According to Dogiel the axons of both types, after exhibiting their peculiar plexiform arrangements course as single axons for some distance and finally divide in the manner of a *T* or *Y* into central and peripheral fibers, a point which could not be verified in the relatively thin sections with which I worked. It was, however, chiefly the origin of the axon from several roots, the splitting of the axon or its roots, and the formation of plexuses in its course which most needed confirmation; the final division of the axon as described by Dogiel agrees so well with our former knowledge of the ganglion that it may safely be accepted. The cells included in groups 3 and 4 are fully as numerous as those in group 2; and if one might be permitted to make a very rough

estimate they might be said to represent together about 3 per cent of the total number in the spinal ganglia of the dog. In the horse, according to v. Lenhossék, they are very much more numerous.

5. Cajal's 'fenestrated' cells are characterized by the presence of excavations in their substance. These are most commonly found in the neighborhood of the origin of the axon, where they often cause a U-shaped mass of protoplasm to be raised from the surface of the cell. The axon usually arises from the summit of such a loop. Fig. 8 shows a simple cell of this kind and fig. 9 a more complicated one. These excavations are filled with small cells, 'subcapsular or satellite cells.' It is only in the poorer of the pyridine silver preparations, however, that these satellite cells are stained. Dogiel was unable to find the fenestrated cells in his preparations—and concluded that Cajal had seen and wrongly interpreted certain cells of our type IV.

Indeed it seems that among his fenestrated cells Cajal has figured and described some which more properly belong with the cells of the preceding variety. There is, however, a group to which the term 'fenestrated' properly applies, such as those seen in figs. 8 and 9. In these the network is formed by protoplasmic loops, while in figs. 5, 6 and 7 the axon is itself broken up to form the network. In the fenestrated cells the axon is usually smaller than the protoplasmic loops from which it arises, while in the other varieties the size of the fibers forming the network is always smaller than that of the axon and depends upon the number of such fibers into which the axon has been split.

In addition to the cells of the varieties just enumerated, one bipolar and two multipolar cells were seen in the sections of the spinal ganglia of the dog. Fig. 10 represents a multipolar cell with short, club-shaped processes, and fig. 11 a cell of Dogiel's type XI, which, according to his more complete pictures, possesses many medullated processes which divide repeatedly, breaking up into fine branches with expanded ends.

According to Nageotte ('07) the fibers ending in end bulbs resemble those seen in large numbers in transplanted ganglia and are therefore to be regarded as being the product of regenerative

activity in the neurone. Cajal ('07) accepts this interpretation and Bielschowsky ('08) extends it to the fenestrated cells and to the cells whose axons have plexuses intercalated in their course. On this hypothesis one would expect to see an increase in the number of such cells after the division of the associated nerve. With this in mind the left sciatic nerve was cut in four dogs and after one month the associated spinal ganglia were prepared by the pyridine silver technique. The results of these experiments were entirely negative. There was no increase in the number of fine fibers ending in end bulbs nor were any other of the peculiar cell types seen in the normal ganglia increased in number. Since the division of the axons might be expected to be as efficient as any stimulus in producing regenerative changes in the neurone, these experiments, so far as they go, speak against the interpretation of these new cell types as the expression of a slow regeneration constantly going on in the normal ganglia. Other experiments are in progress with the purpose of making a more complete test of the hypothesis of Nageotte.

The axons of the small cells

In concluding his section on the cerebro-spinal ganglia in 'Plasma und Zelle,' Heidenhain ('11) says:

> Es würde gewiss für die Physiologie von grosser Bedeutung sein wenn wir behaupten könnten, das wir mit der Anatomie der cerebrospinalen Ganglien im reinen sind. Dies ist jedoch nicht der Fall. Erstlich ist der Ursprung der erwähnten afferenten sympathischen Fasern leider nicht näher bekannt . . . . Und zweitens befindet sich nach den Zählungen von Gaule und Lewin, ebenso von Bühler in den Ganglien eine ausserordentliche Ueberzahl von Zellen deren Fortsätze wir noch nicht kennen.

It is with this second problem that we wish now to deal. The numerical excess of spinal ganglion cells over medullated afferent fibers is well established as table 1 shows, although in most cases it is not so great as that found by Gaule and Lewin in the 32d spinal nerve of the rabbit.

Hatai ('02) by a separate enumeration of the large and small cells of the (IV C, IV T and II L) spinal ganglia of the white rat,

TABLE I

Ratio of spinal ganglion cells to dorsal root fibers

AUTHOR	ANIMAL	NERVE	NUMBER OF GANGLION CELLS	NUMBER OF MEDULLATED AFFERENT NERVE FIBERS
Gaule and Lewin ('96)......	Rabbit	I Coc.	20,361	3,173
Hatai ('02)..................	White rat	VI C	12,200	4,227
Hatai ('02)..................	White rat	II L	9,442	1,644
Ranson ('08).................	White rat	II C	7,721	2,472

showed that the small cells constitute about 60 per cent of the total number, while Warrington and Griffith ('04) working with the II C. spinal ganglion of the cat estimated the small cells as constituting 70 per cent of the total number. Now we have shown in a former paragraph that the axons of these small cells are non-medullated and it is therefore clear that they could not be taken into consideration in the enumeration of the afferent fibers represented in table 1 based as it was in every case upon a differential myelin sheath stain. It is to these non-medullated fibers, the axons of the small spinal ganglion cells, that we are to look for the explanation of the discrepancy between the number of spinal ganglion cells and *medullated afferent fibers*. If a count of the *afferent axons* were made, the number would probably closely approximate that of the spinal ganglion cells.

We have shown in fig. 1 how these non-medullated fibers arise from the small cells, in fig. 2 how they divide dichotomously into a thin fiber directed toward the dorsal root and a slightly thicker one directed toward the nerve. These branches unite themselves into bundles of fine black fibers which course longitudinally through the ganglion—along with the medullated fibers having an analogous origin from the large cells. These bundles of non-medullated fibers can be followed into the dorsal root to which they give an appearance wholly different from that of the ventral root. Similar bundles of non-medullated fibers can be followed into the nerve. Fig. 12 shows the point of union of the ventral and dorsal roots to form the mixed nerve. It can be seen at a glance that the composition of the dorsal root (*a*), as it streams out of the spinal ganglion to unite with the ventral root (*b*) differs

markedly from the latter because of the bundles of fine black fibers which it contains. One can see this contrast in a striking way where the bundles from the two roots decussate as fibers from the ventral root run into the dorsal ramus (*d*) and others from the dorsal root into the ventral ramus (*c*). Fig. 13 represents a portion of this decussation under higher magnification. In the center is a pure bundle of medullated fibers derived from the ventral root, while the fibers taking the other direction are derived from the dorsal root, and of these some are medullated but more are non-medullated.

It has not been possible to show that no non-medullated fibers run into the nerve from the ventral root, but if present they are in very small number. The majority of such fibers seen in the nerve can be directly traced into the spinal ganglion. Nor has the contribution of the ramus communicans to the non-medullated fibers of the nerve been investigated, but even this must be small compared to the enormous numbers coming through the dorsal root.

As to what ultimately becomes of these fibers, there are as yet no observations. That the central branches of the non-medullated axons enter the spinal cord, there can be no doubt, but their distribution within it has not yet been investigated. That their peripheral branches run for long distances in the nerve has been shown in a previous paper. They have been demonstrated in the sciatic nerve of man, dogs, cats, rabbits and rats (Ranson, '11). Figs. 14 and 15 have been drawn with the aid of a camera lucida from adjacent sections of the same human sciatic nerve, the one (fig. 14) prepared according to the Pal-Weigert technique and the other (fig. 15) by the Cajal silver method. The magnification is the same in both instances. Great care was exercised not to decolorize any medullated fibers in differentiating the Pal-Weigert preparations and the field from which fig. 14 was drawn was chosen because it exhibited the maximum number of small medullated fibers. In the Cajal preparation (fig. 15) the colorless rings represent the myelin sheaths, within which are lightly stained axons. In the interspaces between these medullated fibers are enormous numbers of small black axons directly imbedded

in the connective tissue from which they are only separated by a thin neurilemma. The number of axons medullated and non-medullated which can be seen in the Cajal preparation far exceeds the number of myelin sheaths demonstrated by the Pal-Weigert method.

In summing up this point it may be said that the small cells of the spinal ganglion exceed in number the large cells, that their axons are non-medullated and divide after the manner of a *T* or *Y* into a central and a peripheral fiber. The former runs into the spinal cord and the latter can be followed for long distances in the spinal nerves, but the ultimate distribution of neither the centrally nor peripherally directed branch has yet been determined.

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PLATE 1

EXPLANATION OF FIGURES

The drawings are, with the exception of figs. 14 and 15, free hand sketches from pyridine silver preparations of the spinal ganglia of dogs. Figs. 14 and 15 are camera lucida tracings from a human sciatic nerve, the section from which fig. 14 was drawn having been prepared by the Pal-Weigert method, and that from which fig. 15 was drawn by the Cajal method.

1 Small and medium sized cells with non-medullated axons. Non-medullated fibers black, medullated fibers gray.

2 Bifurcation of a medullated fiber and four non-medullated fibers. The latter are fine and black with a triangular expansion at the point of bifurcation, the former is large and grey (in the preparation light yellow) with a constriction at the point of bifurcation.

3–4 Collaterals ending in end bulbs.

5–6 Cells whose axons are split to form a plexus.

7 A cell whose axon arises by three roots which form a plexus out of which the axon is formed

8–9 'Fenestrated' cells.

10 Multipolar cell.

11 Cell of Dogiel's type XI.


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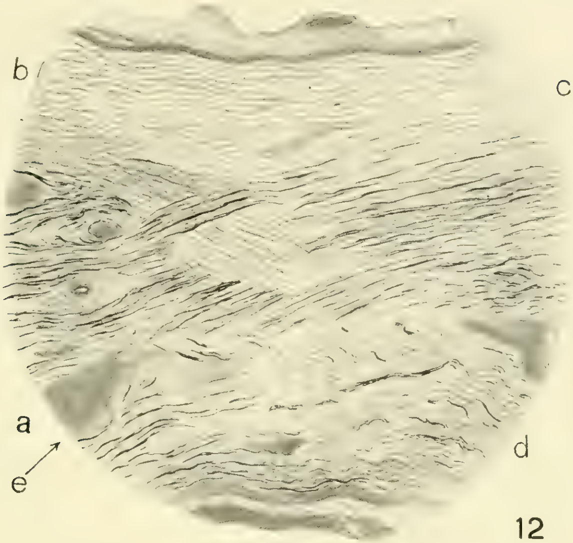
PLATE 2

EXPLANATION OF FIGURES

12 Nerve just distal to spinal ganglion; *a*, dorsal root just outside the ganglion; *b*, ventral root; *c*, ventral ramus of nerve; *d*, dorsal ramus of nerve; *e*, an arrow points to a central bundle of ventral root fibers running into the dorsal ramus.

13 Higher magnification of the central portion of fig. 12. The central bundle is derived from the ventral root and contains only medullated fibers; the fibers passing in the other direction are from the dorsal root. A large part of these are non-medullated.



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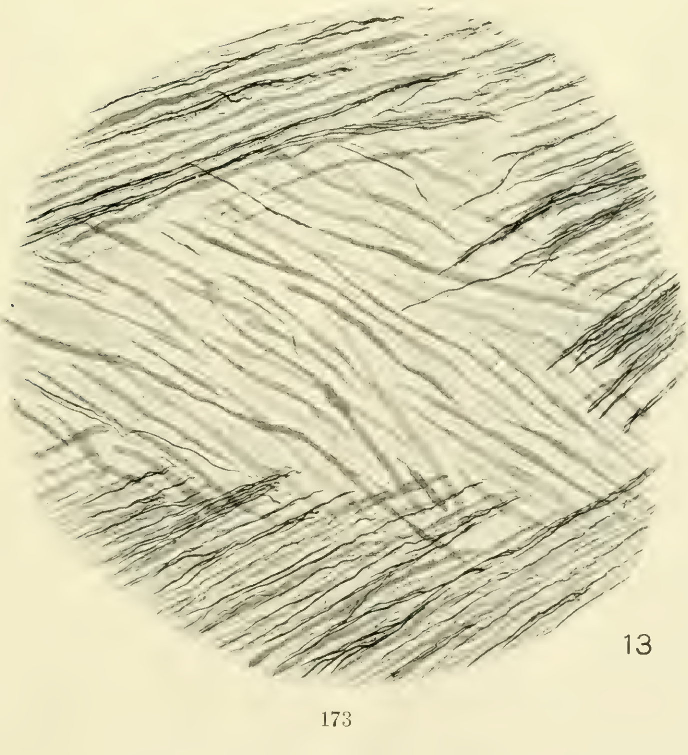

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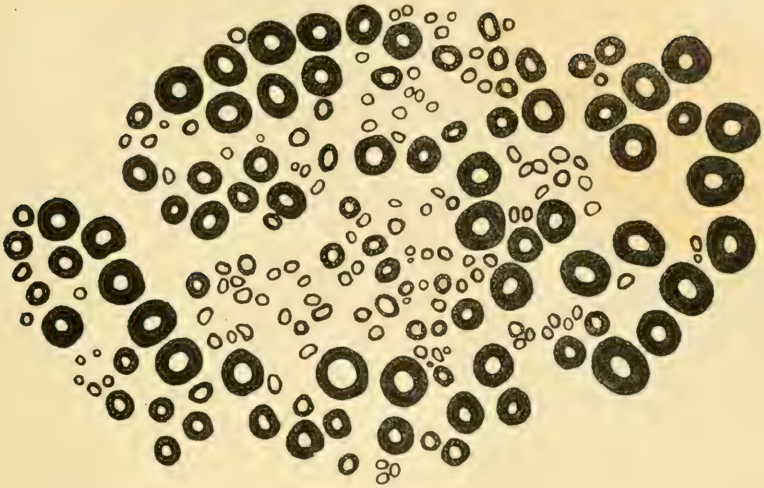
PLATE 3

EXPLANATION OF FIGURES

14 Human sciatic nerve in cross section. Myelin sheaths as black rings.

15 From same nerve as fig. 14, axons black, myelin sheaths colorless. Non-medullated fibers in the interspaces between the medullated ones.



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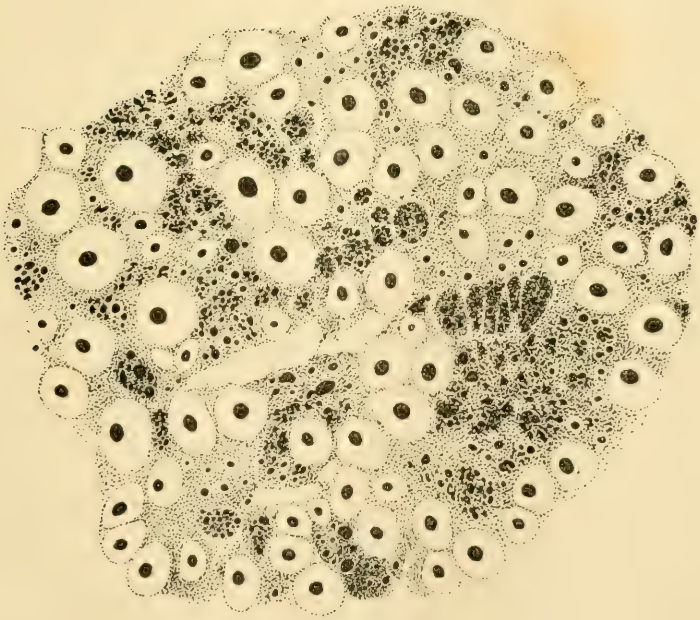

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THE OLFACTORY TRACTS AND CENTERS IN TELEOSTS

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ONE HUNDRED AND FORTY-TWO FIGURES

CONTENTS

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I. INTRODUCTION

The information here added to that heretofore existing in the literature is derived largely from a study of the olfactory apparatus in the carp, Cyprinus carpio (L). The olfactory apparatus is highly developed in the cyprinoids which, therefore, lend themselves readily to its study. For the elucidation of difficult points, however, comparison has been made with Weigert and Golgi sections of the brains of the pike, Lucius lucius (L), the goldfish, Carassius auratus (L) and the catfish, Ameiurus nebulosus (Le Sueur).

I am indebted to Prof. C. Judson Herrick for helpful suggestions and criticism in every phase of the work, together with the opportunity to use his unexcelled neurological library and his personal material. Prof. R. R. Bensley likewise placed at my disposal all the facilities of the department, including the services of the artist, Miss Katharine Hill, who has drawn, with the most painstaking care, the larger portion of the figures. Through the kindness of Prof. Charles Brookover, of Buchtel College I have been able to examine preparations of the brain of Amia calva and to secure well preserved material for most of the Ramón y Cajal impregnations. Acknowledgments should also be made to Prof. B. G. Wilder of Cornell University, at whose suggestion this research was undertaken, and to Prof. S. H. Gage of Cornell University and Prof. E. L. Mark of Harvard University, in whose laboratories important parts of this investigation were conducted.

MATERIAL AND METHODS

The material used consists of the following eighty-three series of sections of the carp brain.

I. Weigert method

Inasmuch as the method used varies somewhat from that usually followed, and since it is very successful, it will be briefly outlined here. The fish are killed in a mixture of ether and water, the brain removed immediately and placed in 4 per cent formaldehyde for at least forty-eight hours. It is then washed in running water for a few hours and placed in Müller's fluid at a temperature of 40° C. for from eight to fourteen days. The fluid is changed every second day during this period. The brain is next washed, dehydrated, cleared in carbol-xylene and embedded in paraffine. After the removal of the paraffine from the mounted sections the slides are placed for twelve hours in a half-saturated solution of copper acetate, stained three to four hours in Weigert's hematoxylin, and differentiated in 2½ per cent potassium ferricyanide with the addition of 2 per cent borax. Pal's modification was tried but rejected, as it was found that sections on the slide could not be evenly differentiated; moreover, the method outlined gives rather better results for the work in question, as it brings out the unmedullated tracts and the cell groups, which the Pal modification does not. There were stained according to this method:

Two series transverse sections of the entire brain.
Two series sagittal sections.
Two series frontal sections.
Six series through the olfactory bulbs and crura.

All of these were from carp 35 to 60 cm. in length and were cut at 15 micra. In addition there were prepared one transverse and one frontal series through the entire head of carp of 3 cm. in length. The method followed in this case is as follows; the fish are placed in Müller's fluid, changed every second day, for a month, and then decalcified for another month in Flemming's

stronger fluid, changed each week. From this point on the method is similar to the one first outlined; viz., dehydration, clearing, embedding in paraffine, etc. Medullated fibers and nerves stand out very distinctly after the use of this method. Other methods of decalcification were tried but, as Herrick ('97) points out, the Weigert method will not 'take' after any of the ordinary processes of decalcification. Professor Herrick was kind enough to loan me, in addition, a series of transverse sections through the adult carp brain, stained by the Weigert-Pal method.

II. Chloral hematoxylin and eosin method

Entire carp, 1 cm. in length, decalcified.
Two series transverse sections.
Three series sagittal sections.
Two series frontal sections.

III. Toluidin blue method

The only fixing agent which gave uniformly good results with this stain was Graf's chrom-oxalic for twelve hours. This method was used for the differentiation of areas and the cytological structure of the nerve cells.
One series transverse sections.
One series sagittal sections.
One series frontal sections.

IV. vom Rath method

This was used for the unmedullated tracts, particularly in the olfactory bulbs, where two series of transverse sections were made. The method followed is that given by vom Rath ('95). This consists of fixation in the following solution for three days; saturated aqueous solution of picric acid, 200 cc., 10 per cent platinic chloride, 10 cc.; 2 per cent osmic acid, 25 cc.; glacial acetic acid, 2 cc. Next the specimens are placed in methyl alcohol for 15 minutes, 0.5 per cent aqueous solution of pyrogallic acid for two days, dehydrated for two weeks in the dark, cleared in carbol-

xylene, embedded in paraffine and sectioned at 5 micra. This method is of particular value in tracing the course of the nervus terminalis.

V. Gold chloride method

This was used to bring out the unmedullated fibers, particularly in the olfactory bulbs; it is not of great value, however, owing to its low penetrating power. The method of fixation in formalin as given in Hardesty's 'Neurological technique,' was followed with the best results. Two series of sections of the bulbs were prepared by this method.

VI. Golgi method

Individuals two to five centimeters in length.
- Five series transverse sections.
- One series frontal sections.

Individuals twenty-five to forty centimeters in length.
- Six series transverse sections.
- One series sagittal sections.
- Nine series cut in various oblique planes.
- Fourteen series of the olfactory bulbs cut in various planes.

Two different methods were followed, both of which gave good results. With most of the Golgi material the fish were killed, the brains removed and placed for three to five days in a mixture of two parts of 3 per cent aqueous solution of potassium dichromate and one part of 1 per cent aqueous solution of osmic acid. Next they were rinsed in a $\frac{3}{4}$ per cent solution of silver nitrate in which they may remain indefinitely but are ready for dehydration and embedding in celloidin after two to four days. The second method was used for brains fixed in 4 per cent formaldehyde.

After fixation for forty-eight hours the brains were washed in running water for twenty-four hours and then placed in a 3 per cent aqueous solution of potassium dichromate at a temperature of 40° C. for six to ten days. They were next placed in the osmium-dichromate mixture and treated as outlined for the first

method. In addition to the series noted above, Professor Herrick very kindly loaned me ten series of the brains of young carp cut in various oblique planes. The Golgi method was used chiefly for the study of the different neurones and the course, with particular reference to the direction, of the fiber tracts.

VII. Ramón y Cajal method

Two series transverse sections.
Three series frontal sections.
Five series sagittal sections, two of these partly oblique.

Some difficulty was experienced in getting good preparations, the following method giving the best results. Whole brains are fixed in 95 per cent ethyl alcohol, washed for two hours in running water, placed in a 1 per cent aqueous solution of silver nitrate at a temperature of 35° C. for three to five days, washed in distilled water, transferred to a 1 per cent aqueous solution of hydroquinone for twenty-four hours, washed in running water for twenty-four hours, dehydrated, cleared in cedar oil, mounted in paraffine and cut at ten to fifteen micra. Fixation in neutral or acid formalin gave poor results.

II. ANATOMY

The names applied to the different fiber tracts and cell areas have, so far as is consistent with their morphology, been taken from the literature. In a few cases such terms have been used in a sense slightly different from that assigned them by the original authors; whenever such is the case the fact has been noted. In several cases inappropriate terms of long use have been retained owing to their familiarity and common use. Where, however, a term is lacking in the literature, or where a previously used term is greatly at variance with the morphology, a new name has been selected. In this case the endeavor has been, as far as possible, to make such new name descriptive of the relationships involved, or else suggestive of a homologous structure

in the nervous anatomy of higher forms. In the case of fiber tracts the customary methods of neurological nomenclature have been followed, viz., the application of a term which will include in itself full information as to the origin and termination of the fibers as well as their direction; as for example, the tractus intermedio-habenularis, originating in the nucleus intermedius and terminating in the habenula.

1. PERIPHERAL OLFACTORY APPARATUS

a. The olfactory capsules

The olfactory apparatus in the carp consists of the olfactory capsules with their lamellae, open to the exterior through two apertures; the olfactory nerves, bulbs, crura, centers in the cerebral hemispheres, epithalamus, medithalamus and hypothalamus, to which may be added the motor connections common to the olfactory and gustatory senses, etc. These latter will not be considered in this article.

Gross morphology. The two external apertures of the capsules are in close proximity, one rostro-medial of the other. They are separated by a grooved flap of skin so shaped that in forward movement water will be driven into the more rostral aperture. The lateral aperture opens caudally for the exit of water from the cup. Inside the capsule, and running caudo-laterally from the rostro-medial aperture, is a median ridge from which the lamellae radiate on either side and at its caudo-lateral end.

Microscopic anatomy. The lamellae are covered by the epithelium of the olfactory mucous membrane, consisting of the typical nervous olfactory cells, and the supporting cells. Goblet cells are particularly numerous in the epithelium of the central ridge, which is also slightly thicker than that of the lamellae (fig. 5). It resembles closely the respiratory epithelium of the Schneiderian membrane of mammals, as distinguished from the olfactory portion. It is probable, therefore, that there are found here two varieties of epithelium, similar to the condition in higher forms; an olfactory, concerned with smell and a respiratory, concerned, in this case, with the water current.

Innervation. From the olfactory cells arises the unmedullated fibers which, passing through the lamellae, form the olfactory nerve. Medullated fibers penetrate the central ridge, ending immediately underneath the epithelium (fig. 5). Such an innervation has been described in no other anamniote (Sheldon, '08 b), although it has long been known that in mammals, particularly in man, such fibers take part in the innervation of the nasal mucous membrane.

In 1903 Rubaschkin demonstrated their presence in birds. Practically all Amphibia and many fishes have been studied with reference to this point, but in no case have medullated fibers been demonstrated beyond doubt, although Aichel in 1895 believed that he found something of the kind in embryo teleosts. In six Weigert series through the olfactory capsules, bulbs and crura of the adult carp it has been possible to demonstrate the presence of medullated fibers in the tunica propria of the Schneiderian membrane, part of which evidently distribute to the epithelium, as they can be traced to the membrana propria itself. These latter probably end in free nerve terminations, as there are no special organs developed. Part of the fibers entering the tunica propria join the bundles of unmedullated fibers and apparently run to the mucous membrane of the lamellae with them. The remainder of the medullated fibers innervate the skin about the nasal capsule.

All of these medullated fibers are derived from the supraorbital trunk, which is made up of general cutaneous fibers from the Gasserian ganglion (nervus ophthalmicus superficialis trigemini) and sensory fibers from the facial (nervus ophthalmicus superficialis facialis). This latter nerve is composed partly of fibers from the dorsal lateralis ganglion, and partly of visceral sensory fibers from the geniculate ganglion. The fibers entering the tunica propria are certainly not acustico-lateral, since no canal or pit organs are developed in connection with the epithelium; the fibers are also smaller than are the lateralis fibers. They may, therefore, be either general cutaneous or visceral sensory, with the preponderance of evidence in favor of the former. This is due, in part, to the fact that in birds and mammals such innerva-

tion is trigeminal and partly because the weight of evidence in the teleosts is against the supposition that visceral sensory fibers are present in this region. Part of the branch entering the tunica propria goes to the skin, as already noted; the number of general cutaneous fibers in the supra-orbital trunk is much greater than the number of visceral sensory. If there are visceral sensory fibers going to the mucous membrane, they must be unspecialized, as there are no taste buds present; there is not the slightest evidence, however, that such fibers are here present. In their course from the supra-orbital trunk to the tunica propria the medullated fibers pass partly between the two bundles of the olfactory nerve and partly directly laterad into the median ridge.

Young gold fish and cod were studied with reference to the presence of medullated fibers in the mucous membrane, but none could be demonstrated. This may have been due, particularly, in the case of the gold fish, to the fact that the individuals were immature, as such fibers could not be found in young carp.

As the main current of water would be forced along the ridge thus innervated by general cutaneous fibers, it is probable that their function is that of tactile response for solid substances in the water or else with respect to the strength of the water current or both (see also Kappers, with respect to the 'Oralsinn,' and Sheldon, '09 b, on 'Chemical Sense').

b. The olfactory nerve

The olfactory fibers gather from the different lamellae in two main bundles. In general, the medial bundle is derived from the more rostral lamellae, while the lateral is derived from the more caudal. The fibers of the two bundles distribute to all parts of the rostral and lateral surfaces of each bulb, the lateral bundle causing a protuberance on the dorso-lateral surface of each bulb as shown in figs. 1, 6 (*a*). There is a quite general crossing of the fibers of the two bundles before they reach the bulb so that fibers from each reach all parts of the bulb (fig. 123). Apparently, however, the lateral bundle is more especially associated with the tractus olfactorius lateralis and to a somewhat less extent with the

tractus olfactorius ascendens, while the medial bundle is most closely associated with the tractus olfactorius medialis. The olfactory bulb is closely applied to the caudo-mesal face of the capsule so that the nerve itself is very short, although individual fibers may be of some length.

c. Ganglion of the nervus terminalis

Lying between the two bundles of the olfactory nerve from the lamellae to the bulb are a number of large scattered ganglion cells forming the ganglion of the nervus terminalis. In the adult carp these cells are most numerous near the bulb and are apparently about a hundred in number. This is less than is the case in Amia as described by Brookover ('08, '10). Neurites from these cells run mesad to form a bundle of fibers on the mesal aspect of each bulb (figs. 7, 123, 124).

2. THE TELENCEPHALON

a. Olfactory bulb and crus

The olfactory bulb is ellipsoid in shape, about 1.5 mm. long and 1 mm. thick, in a 40 cm. carp. Rostrally and laterally it is covered by a mass of olfactory nerve fibers as noted above. At the rostral end of the bulb a circular constriction appears externally, separating the bulb proper from the olfactory nerve proper, which rostro-lateral to the constriction spreads out over the olfactory capsule. Caudally the bulb tapers down to the small, elongated crus on which it is borne in the cyprinoids. This is from three to four centimeters long in a 40 cm. carp, extending from the bulbs to the cerebral hemisphere (fig. 1). In young fry the bulbs are closely apposed to the hemispheres; but since the cranium grows faster than the brain as a whole, the crura elongate. Each crus is a hollow tube, the base of which is formed by the tracts connecting the bulb and hemisphere (figs. 2, 22, 23). Dorsally is an epithelial roof, a rhinotela, which is simply a rostral prolongation of the tela, or so-called pallium of the hemispheres, consisting of a layer of ependyma and one of pia. This covering arches over the

solid base of the crus at its caudal end as shown in fig. 23, gradually decreasing in extent rostrad (fig. 22) until it forms only a roof for the trough-like cavity below. This cavity is morphologically a part of the ventricle of the hemispheres, extending, even a short distance into each bulb, as is the case with most vertebrates (Wiedersheim, '02).

Internal to the layer of olfactory nerve fibers occurs the formatio bulbaris, formed chiefly by the glomeruli. The glomeruli are of the usual type, consisting of the terminal end-brush of olfactory nerve fibers, mingled with the dendrites of mitral cells, chiefly. The central and mesal portion of the bulb is made up of a mass of cells, the nucleus olfactorius anterior, the lobus olfactorius anterior of Goldstein.

According to Golgi preparations, neurones of several different types are found in the olfactory bulb. The most conspicuous are the large cells with short, thick, many branched dendrites, the mitral cells (figs. 8 to 12). These are irregular in form and are situated largely in the peripheral portion of the bulb, with their long axes approximately parallel with the surface as figured by Johnston, Catois, etc. The mitral cells are very irregular in form; pyramidal, stellate and goblet shapes being the most numerous. The dendrites of these cells, as already mentioned, break up in the glomeruli and there come into relation with the terminals of the olfactory nerve fibers. Their neurites form the majority of the centripetal fibers of the tractus olfactorius lateralis and tractus olfactorius medialis. A dendrite of a mitral cell will often enter, also, into relation with one of the cells of the nucleus olfactorius anterior, usually a fusiform or stellate cell. The smaller cells of the bulb are more nearly central in position and make up most of the nucleus olfactorius anterior. Fusiform and stellate cells are the most numerous of these, with occasionally a pyramidal or goblet-shaped cell (figs. 13 to 20). The stellate cells, particularly of the types shown in figs. 14 and 17, are the most common, and are situated near the center of the bulb, with their many processes extending fan shaped toward the periphery, where many of them enter glomeruli (Johnston, '98, fig. 1). Other processes of these cells enter into relation with other cells

of the nucleus olfactorius anterior. It is certain that a few of the stellate cells send their neurites into the hemispheres, but such could not be demonstrated with certainty for all. Many fusiform cells of the type shown in fig. 13 lie near the center of the bulb with two processes extending to either margin. These cells likewise send their neurites to the hemispheres (fig. 21). Cells of the types shown in figs. 15, 16, 18, 20 may be found in any part of the nucleus olfactorius anterior, with their processes extending nearly equally in all directions. Small granule cells, of the type shown in fig. 19, are common in the center of the bulb, where they apparently function as association cells, as no neurites entering the crura could be demonstrated. The mitral cells undoubtedly correspond with the mitral cells of all other vetebrates, so far as studied; there is some question, however, regarding the comparative morphology of the smaller cells throughout the vertebrate series. Apparently, as is shown also by Johnston, the stellate cells in lower vertebrates are connected with the glomeruli and hemispheres, much as are the mitral cells; as an ascent is made in phylogeny, however, these cells may either disappear or may metamorphose into mitral cells. The typical stellate cells of the carp as shown in figs. 14, 17, are undoubtedly similar to the stellate cells of the granular zone of Acipenser, as described by Johnston; there is the same relation to the glomeruli, the position in the bulb is the same, and the central processes take the same course. Fusiform cells of the type shown in fig. 13 are probably the homologues of Johnston's spindle cells of the granular zone, although no neurites were traced from these cells into the crura. The type found in figs. 15 and 20 probably corresponds to Johnston's cells with short neurites, Golgi type II cells. Cells of Cajal were not identified with certainty. The granule cells of the carp are apparently simply intrinsic association nerve cells, differing, therefore, from the granule cells of Acipenser.

The fiber tracts of the olfactory bulb will be taken up later, in connection with the fiber systems of the cerebral hemispheres.

b. The cerebral hemispheres

(1) Gross morphology. The cerebral hemispheres are of the typical teleostean type (figs. 1 to 4). They consist of paired solid basal lobes which contain chiefly the secondary olfactory centers, continuing caudo-ventrally over the optic chiasma as the pedunculi thalami, or praethalamus of C. L. Herrick. Dorsally and laterally, these are covered by a membranous roof, the so-called pallium, composed of adjacent layers of ependyma and pia. This tela is continuous rostrally with the membranous roof of each olfactory crus, the separation into two parts occurring just at the rostral margin of the basal lobes. This tela is attached at the ventro-lateral margin of each hemisphere, at which point its pia becomes continuous with that of the base of the brain, while its ependyma is reflected over the basal lobes (figs. 1, 2, 3, 4, 34). Immediately mesal to the attachment of the tela occurs a fissure, the fissura endorhinalis (figs. 4, 24, 25, etc.). This is the sinus rhinalis of Kappers ('06), the fovea endorhinalis externa of Kappers and Theunissen ('08), the fovea limbica of Goldstein and Edinger, the fissura ectorhinalis of Owen ('68), the fissura endorhinalis of many authors. This fissure holds a constant position throughout the vertebrate series, separating in the higher forms the basal olfactory centers from the pyriform lobe; it likewise bears a constant relation to the tractus olfactorius lateralis, as will be noted later.

The ventricle of the forebrain consists of the open space between the tela and the basal lobes. This forms a large, but shallow cavity, excepting between the two basal lobes where it is of some depth (figs. 24, 34, 35, etc.). It extends caudally to the velum transversum. Caudal to this velum, occurs a much convoluted epithelial sac extending rostrally over the tela proper; this is the dorsal sac, and is an evagination of the membranous wall of the diencephalon (fig. 68). Ventral to the velum transversum, the forebrain ventricle passes over into the third ventricle or diencephalic cavity.

Each basal lobe is separated by ependymal sulci on the dorsal, lateral and mesal surfaces into regions with characteristic internal

structure and fiber connections (figs. 2 and 3). The deepest of these is the sulcus ypsiliformis, which arises from the ventrolateral border about three-fourths of the distance back from the rostral pole of the basal lobe, ascends to the dorsal surface and here divides into an anterior and a posterior limb, which enclose a central eminence. This eminence contains the palaeostriatum and the primordium hippocampi, the latter covering the dorsal surface of the palaeostriatum, especially on its mesal border. The posterior limb separates the posterior pole from the rest of the hemisphere; the anterior limb separates the central eminence from the tuberculum anterius and the tuberculum laterale, these comprising a part of the nucleus olfactorius lateralis. The remainder of the lateral olfactory nucleus is the nucleus pyriformis of the posterior pole.

The anterior limb of the sulcus ypsiliformis corresponds fairly closely with the sulcus palaeopallio-epistriaticus of Thynnus and the fovea endorhinalis interna of Amia, as described by Kappers and Theunissen ('08).

On the mesal aspect of each basal lobe, extending for almost the whole length of the lobe is a well defined sulcus of great morphological importance which has been ignored by other writers on the brains of fishes. It forms the dorsal boundary of the precommissural body and has some points of resemblance with the fissura limitans hippocampi (C. Judson Herrick, '10) in Amphibia and Reptilia, the fovea septocorticalis (Kappers and Theunissen) in Rana, and the fissura arcuata of Gaupp, with which, however, it is not fully homologous, as will appear beyond. It will be designated sulcus limitans telencephali.

Ventrally of this furrow lies the corpus precommissurale, termed the epistriatum by Kappers ('06), the lobus olfactorius posterior, pars medialis, by Goldstein, etc.

An examination of fig. 4 shows that the fissura endorhinalis on the ventral surface of the hemispheres forms an open V. It first appears rostrally at the point where the olfactory tract joins the hemispheres (fig. 24), gradually extending laterally until the base of the sulcus ypsiliformis is reached, whence it turns medially again. For the whole of its extent the tractus olfactorius lateralis

lies immediately dorsal, giving off fibers to the nucleus olfactorius lateralis and nucleus pyriformis. Lateral to the caudal end of the fissura endorhinalis lies the nucleus teniae of Edinger, Kappers and Goldstein.

(2) *Nuclei.* The basal lobes are entirely separate, excepting ventrally, where they are joined by the lamina terminalis, which runs rostrally from the region of the optic chiasma. At a point approximately two-thirds distant from the rostral margin of the hemispheres, there lies embedded in the lamina terminalis, the large anterior commissure, connecting both lobes (figs. 34 to 61). Rostrally, the lobes overhang the olfactory tracts for a short distance (fig. 24), while caudally the hemispheres, spreading laterally over the optic tracts, are partly covered by the optic lobes (fig. 76).

The basal lobes contain, in teleosts, the secondary olfactory centers, one or more tertiary centers and the so-called corpus striatum, here designated the palaeostriatum. In the carp this receives, throughout most of its extent, secondary olfactory fibers.

(a) Corpus precommissurale. Extending from the rostral end of the hemispheres caudally into the diencephalon is a column of cells, bordering the medial cavity on either side. Its dorsal limit is indicated by the sulcus limitans telencephali and it is bounded laterally, throughout most of its more caudal portion, by the palaeostriatum.

This is the corpus precommissurale; the area olfactoria posterior medialis and epistriatum of Kappers, '06, but not of Kappers, '08, where this name is applied to the primordium hippocampi; the lobus olfactorius posterior, pars medialis of Goldstein; 'vordere nucleus,' partly, of Bela Haller. At the rostral end of the hemisphere this nucleus is largely ventral (fig. 25); toward the anterior commissure, however, it increases in dorso-ventral extent covering practically all the mesal surface of each hemisphere (fig. 35). The interposition of the fibers of the commissure separates the nucleus into two parts, a dorsal passing above the commissure, and a ventral composed of cells lying between its fiber systems (figs. 35, 36, 38, 55, 56, 61). Caudally of the anterior commissure, these two divisions of the nucleus remain distinct, one continuing

ventrally, close to the median cavity, while the other remains dorsal, meeting the lateral olfactory area in the polus posterior of the hemisphere, and then continuing caudally under the habenula. This forking column of cells is, as will be brought out more clearly later, the morphological equivalent of the precommissural body or paraterminal body of Elliot Smith in mammals and reptiles, and is, therefore, here termed the corpus precommissurale. The rostral portion of the nucleus corresponds morphologically to the rostral part of the ganglion mediale septi of Gaupp, or the area precommissuralis septi of Kappers and Theunissen in the frog, and is called, therefore the nucleus medianus (fig. 25).

The portion of the nucleus extending into the commissure is simply the bed of the anterior commissure of Elliot Smith in reptiles and mammals and is called, therefore, the pars commissuralis. The arm of the precommissural body arching over the commissure presents points of resemblance to the pars fimbrialis septi of Kappers and Theunissen in the frog. It is here called the pars supracommissuralis (figs. 35, 36, 38, 55, 56, 61). Its extension caudad behind the commissure joining the lateral olfactory area is named the pars intermedia (figs. 66, 67, 68, 70). The commissure bed passes immediately caudad into a nucleus of small cells, bordering the ventricle, which is here termed the nucleus preopticus (figs. 61, 66, 67, 68, 70, 73, 76, etc.). This is composed of several different cell groups which will be taken up in greater detail later.

All parts of the corpus precommissurale appear very discrete in toluidin blue preparations. In the nucleus medianus, the cells are closely packed, but are arranged in groups or islands (figs. 25, 26). (See Calleja, '93.) Usually a clear zone of few cells surrounds the precommissural body particularly dorsally and laterally (fig. 38). In the pars supracommissuralis the cells are less closely packed (figs. 38, 46, 56), and have lost the island arrangement. The grouping in the pars commissuralis, is largely dependent on the position of the fiber bundles of the anterior commissure; the cells are, however, fairly evenly distributed (figs. 38, 56).

In the rostral part of the commissure bed is found the group of cells in which terminate the fibers of the nervus terminalis. The pars intermedia of the corpus precommissurale consists of a narrow column of cells, fairly closely packed and forming a distinct band across the ventral portion of the posterior pole of the hemisphere (figs. 66, 67, 68, 70). Its morphological relationships are obscure.

In Golgi preparations of different parts of the precommissural body some of the cellular relations are brought out more fully. In the nucleus medianus the cells are fairly large, fusiform, pyramidal or ellipsoid in shape, with almost all of their processes coming from the ends of the perikaryon as shown in figs. 28 to 31. A large proportion of these cells give rise to the fibers of the tractus olfactorius ascendens. The neurites are very delicate, possessing granular enlargements along their course. Smaller cells with a number of short, root-like dendrites and a single long neurite extending into the palaeostriatum, are not uncommon (fig. 43). Several varieties of small cells, apparently functioning as association cells, are found also in the nucleus medianus; these are chiefly stellate, or irregularly rounded (figs. 41, 42). In the pars supracommissuralis the cells are smaller; also rather more of the association cells of the type shown in figs. 41, 42 are found. Cells of type shown in fig. 43, sending fibers to the palaeostriatum, are more common than in the nucleus medianus. Many of the cells of this nucleus send their neurites into the tractus olfacto-thalamicus medialis. Such a cell is shown in fig. 40. Small stellate and small pyramidal cells are rather more common than the type illustrated.

(b) Primordium hippocampi. Dorsad of the sulcus limitans telencephali, appearing with especial distinctness rostrally, lies the primordium hippocampi, or nucleus olfactorius dorsalis. Between it and the corpus precommissurale may be seen a slight clear area, devoid of cells. The cells of the primordium hippocampi are rostrally slightly smaller than those of the nucleus medianus, while dorsal to the pars supracommissuralis they are very similar to those of the latter nucleus (fig. 46). Many of them resemble the dorsal cells of the nucleus olfactorius lateralis (figs.

48, 49, 56). The primordium hippocampi receives secondary olfactory fibers from the tractus olfactorius medialis and a few commissural fibers associated with the commissura corporium precommissuralium. The neurites of its cells descend partly with the tractus strio-thalamicus and partly with the tractus olfacto-thalamicus medialis, pars dorsalis. No tertiary olfactory fibers could be traced, in Golgi preparations, either from the lobus pyriformis or the corpus precommissurale to the primordium hippocampi. In Ramón y Cajal preparations, however, mingled with the fibers of the commissura corporium precommissuralium rostrally are a number of unmedullated fibers connecting the nucleus medianus with the primiordium hippocampi. From the conditions in amphibians, reptiles and mammals it seems extremely probable that these represent the tractus area-hippocampalis rectus of Kappers and constitute an association path between the precommissural body and the primordium hippocampi. The morphology of this region will be considered more in detail further on.

(c) Nucleus olfactorius lateralis. Laterally, extending from the extreme rostral end of each basal lobe to the extremity of the polus posterior, lies the lateral olfactory area; the area olfactoria of Edinger, the lobus olfactorius posterior, pars lateralis of Goldstein, area olfactoria posterior lateralis of Kappers ('06), area olfactoria lateralis of Kappers and Theunssen ('08). The nucleus olfactorius lateralis is here divided into itwo parts, both rostral to the sulcus ypsiliformis, and consisting of rather evenly distributed, somewhat scattered cells. The more rostral appears externally as the tuberculum anterius (figs. 2 and 3), while the more caudal presents superficially the tuberculum laterale. The nucleus olfactorius lateralis covers as a cap the entire rostrolateral surface of each basal lobe. At the extreme rostral pole it is restricted to the lateral aspect but passing caudally it gradually spreads dorsally covering the dorso-lateral aspect of each lobe, at the level of the sulcus ypsiliformis (figs. 25, 38).

The lobus pyriformis, so named since it is closely related to the pyriform lobe of mammals, consists dorsally and caudally, of evenly distributed scattered cells very similar to those of the nucleus olfactorius lateralis (figs. 38, 56, 66, 67). Ventrally, imme-

diately lateral to the fissura endorhinalis, a portion of the nucleus pyriformis is specialized to form the nucleus teniae of Kappers, Goldstein, Edinger, the caudal portion of the hypostriatum of Catois, nucleus occipito-basalis of C. L. Herrick. This is a compact nucleus of rather small cells (fig. 57). Caudally it meets the pars intermedia of the corpus precommissurale, both being covered dorsally by the unspecialized cells of the lobus pyriformis (figs. 38, 56, 66, 67, 70).

In preparations by the Golgi method, this region is plainly marked. Throughout the whole lateral olfactory area, near the periphery of the lobes, one finds cells of the same general type, with fine processes, lightly spiny, and with small sized perikarya (figs. 32, 33, 48, 49, 52, 53). The perikarya vary considerably in shape, flask-shaped cells being most numerous, as shown in figs. 32, 33, from the rostral portion of the lateral area, figs. 48, 49 from the dorso-lateral part. Occasionally, small pyramidal cells of the type shown in fig. 53 may be found. Flask-shaped cells are particularly numerous close to the periphery of each lobe, with the rounded margin of the perikaryon directed toward the periphery and most of the processes arising from the mouth of the flask. A cell of this type is shown in fig. 52. Part of these processes extend laterally along the ventricular margin, while the neurite enters the basal forebrain bundle.

The cells of the nucleus teniae vary somewhat from the general type of the lateral olfactory area neurone but are recognizably similar. Many of the cells, as shown in figs. 59, 60, possess perikarya more nearly ovoid than flask-shaped; the processes are fine and bear inconspicuous spines, however, as do the other cells of the lateral olfactory area. Fig. 58 shows a cell nearly pyramidal in shape.

(d) Palaeostriatum. In the central part of each basal lobe is a region called by practically all writers on the teleostean brain the corpus striatum, here termed the palaeostriatum (figs. 25, 38, 56). It is bounded mesially by the precommissural body, dorsally by the primordium hippocampi and on the other sides by the lateral olfactory nucleus. Practically all parts of it receive olfactory fibers of the second order and it is largely, therefore, a

portion of the mesal and lateral olfactory areas. The cells of the central part of this area are very large and conspicuous (fig. 44) and are quite scattered as compared with the cells of other areas of the basal lobes. In series stained by cytological methods, such as toluidin blue or thionin, it is easy to demonstrate that there is a gradual transition from these conspicuous cells to those typical of the lateral olfactory area.

According to the Golgi method, neurones of the central portions of the palaeostriatum appear very large, with comparatively enormous perikarya, and with long, thick, very thorny dendrites (figs. 50, 51). In shape, the perikarya vary from short, flask-shaped to pyramidal (fig. 45).

There is shown in Golgi preparations the same transition between the area olfactoria lateralis and the palaeostriatum, as in toluidin blue or thionin preparations. One may note a gradual change, in passing from the periphery centrally, from the small, flask-shaped cells with rather inconspicuous thorns, to the large cells, with enormous perikarya and thick, thorny processes; moreover, now and then, a cell of the palaeostriatal type will be found close to the periphery, or a small lateral area cell found in the palaeostriatum. A large proportion of the cells of both the lateral olfactory area and the palaeostriatum send their neurites into the basal forebrain bundle, the different parts of which will be taken up later. The neurites of the cells of the nucleus teniae, however, enter the tractus teniae. Many of the cells of these two areas are apparently association cells, functioning not only to bring different parts of the same area, but also adjacent areas, such as lateral olfactory area, palaeostriatum and corpus precommissurale, into relation. Such is apparently the function of some of the cells of the type shown in figs. 50, 51.

The word 'palaeostriatum' is not used in quite the same sense as it is used by Kappers, as will be noted from the preceding discussion. Kappers believes that the palaeostriatum is closely connected with the olfactory apparatus, but receives no somatic sensory connections from the thalamus, which it probably does receive in the teleosts. The term as here used indicates that a structure is found in the teleosts, closely related to the secondary olfactory

centers, and morphologically related to a part, at least, of the corpus striatum of higher forms.

(e) Nucleus commissuralis lateralis. Situated in the ventromedial portion of each basal lobe, in the region of the anterior commissure, at either end of the commissure is a small compact nucleus of fairly large, closely packed cells (figs. 38, 56). No references to it in the literature have been noted; it has, therefore, been termed the nucleus commissuralis lateralis, owing to its location, laterally at the level of the anterior commissure.

(f) Nucleus preopticus. Immediately caudal to the anterior commissure there appears ventrally the recessus preopticus of the third ventricle (fig. 56). Surrounding this, on either side, and passing rostrally insensibly into the pars supracommissuralis, is the nucleus preopticus. This nucleus is composed of cells of two types; at the level of the caudal margin of the hemispheres is a dense mass of cells bordering the median ventricle; its cells are some of the largest in the brain (fig. 71), and are flask-shaped with their bases directed toward the ventricle and most of their processes extending laterally and ventro-laterally (figs. 67, 70, 71). This is here termed the pars magnocellularis of the nucleus preopticus. Rostral to this nucleus, continuous with the pars supracommissuralis, is a nucleus of small cells (fig. 64). This group of cells extends caudally, lateral to the pars magnocellularis, gradually curving around it caudally, thus enclosing the nucleus magnocellularis on three sides. In contradistinction to the nucleus magnocellularis this is called the pars parvocellularis of the nucleus preopticus. To the portions rostral, lateral and caudal to the pars magnocellularis are assigned the suffixes, anterior, lateralis and posterior, respectively (figs. 64, 66, 67, 70, 78). This nucleus extends caudally to the region of the fibrae ansulatae.

In Golgi preparations the pars parvocellularis shows cells of several types, resembling closely the various kinds of small cells of the corpus precommissurale.

The nucleus preopticus was recognized by C. L. Herrick in 1892. Herrick saw both the large and small cells and applied the name nidulus praeopticus to the larger portion of the nucleus; it is probable, however, that his nucleus postopticus contains a por-

tion of the cells here included under the name nucleus preopticus. Bela Haller noted the same group of cells and termed it the nucleus posterior of the forebrain. Johnston ('98) and ('01) found a nucleus bordering the recessus preopticus and termed it the nucleus thaeniae owing to the fact that he observed fibers passing from it to the habenula, and that, therefore, it is ('98) "a nucleus corresponding to the nucleus occipito-basalis of (C. L.) Herrick and the nucleus thaeniae of Edinger" in reptiles. This view is untenable as will be pointed out later. Johnston probably recognized the fact, as he terms it nucleus praeopticus in his 'Nervous System' ('06). Kappers noted the large cells and, following Herrick, named the group, nucleus praeopticus. Goldstein, following the descriptions of Edinger for the brains of reptiles and birds, applied the names magnocellularis and parvocellularis strati grisei to the two components of the nucleus.

(g) Nucleus entopeduncularis. Appearing immediately caudal to the nucleus commissuralis lateralis is a group of very small cells (fig. 65), lying embedded in the basal forebrain bundle (figs. 66, 67, 68, 70). This is the nucleus entopeduncularis of Goldstein.

3. THE DIENCEPHALON

a. Rostral limits

The division of the vertebrate brain into transverse segments, with a clear definition of their limits, is a matter of considerable difficulty, particularly since, as Johnston and C. J. Herrick have pointed out, most of the important morphological centers and fiber connections are arranged in longitudinal columns. The question of the caudal boundary of the telencephalon ventrally is still unsettled, some authors considering the pedunculi thalami, caudal to the anterior commissure, part of the diencephalon, others placing the rostral limits of the 'tween-brain behind the optic chiasma in the adult. Dorsally the caudal margin of the velum transversum has long been considered the limit of the forebrain. Johnston recently ('09) has taken up the subject in some detail and his interpretation is here followed; according to which

the caudal limits of the forebrain include the velum transversum and the optic chiasma. The pedunculi thalami, the praethalamus of C. L. Herrick, are included in the telencephalon, and their centers have already been described (nucleus preopticus and nucleus entopeduncularis).

Most writers on the brains of fishes have, however, included these structures in the diencephalon; in fact even under the interpretation here followed, the pars parvocellularis posterior of the nucleus preopticus extends into the diencephalon, since it reaches caudally to the level of the fibrae ansulatae.

b. Gross morphology

The diencephalon in the carp is of the typical teleostean type. Immediately caudal to the velum transversum, the diatela is thrown into a convoluted folded epithelial sac, extending rostrally over the membranous pallium of the hemispheres, forming the saccus dorsalis, post-velar arch, or Zirbelpolster (figs. 68, 73). This is an extremely vascular structure, formed by the covering of pia mater and a lining, continuous with the ependyma of the third ventricle. Arising immediately caudal to the saccus dorsalis, with the caudal wall of the one practically adherent to the rostral wall of the other, is the epiphysis or pineal body. This is a small elongated tubular organ extending rostrally, suspended in the folds of the dorsal sac. Its epithelium, while an extension of that of the ependyma, is glandular in type. Lying embedded in the membranous wall between the dorsal sac and the epiphysis, is found the commissura habenularum, or commissura superior. At the caudal base of the epiphysis is found the commissura posterior, between it and the tectum opticum.

The diencephalon is commonly subdivided into epithalamus, hypothalamus and thalamus. The latter has been divided by C. J. Herrick ('10), following Ramón y Cajal, into pars dorsalis (sensory correlation centers) and pars ventralis (motor correlation centers). The epithalamus of the carp is distinct; the other parts are so confused that further embryological study will probably be necessary to effect this separation; and the assignment of the differ-

ent nuclei and fiber tracts to these regions in this paper must be regarded as provisional, particularly with respect to the centers lying within, and immediately dorsal to, the lateral parts of the inferior lobes.

The inferior lobes consist of an unpaired pars medialis, which is clearly hypothalamic, and paired partes laterales, the lateral lobes, which apparently belong chiefly to the pars ventralis thalami.

The lobi laterales are widely separated rostrally by the interposed lobus medius, while they meet one another caudal to it. Caudally a furrow appears on the ventral aspect of the lateral lobes, the sulcus mammillaris of Goldstein (fig. 4). The prominence of the lobes mesal to the two sulci, is due to the development dorsally of the corpora mammillaria of Goldstein (fig. 117). Laterally, each inferior lobe shows several lobes and sulci, varying somewhat in different individuals. Rostrally the great size of the nucleus prerotundus and nucleus rotundus causes the development of a slight protuberance, appearing on the outside of the lobe (fig. 3). Further caudally the nucleus cerebellaris hypothalami gives rise to a similar enlargement (fig. 3). The lobus medius consists of the tuber cinereum rostrally, and the pars infundibularis caudally.

Extending ventro-rostrally from the tuber is found the hypophysis, consisting of the two conspicuous solid lobes, separated by a circular constriction; a rostral pars glandularis and a caudal pars nervosa. Ventrally these are separated into symmetrical parts by a longitudinal median furrow (fig. 4). Extending caudally from the caudal margin of the pars infundibularis of the lobus medius is a narrow, thin, glandular, membranous sac, the saccus vasculosus, opening into the infundibular cavity (fig. 4).

The median cavity of the forebrain extends caudally and ventrally between the two pedunculi thalami and thalamus proper, giving rise to diverticula which penetrate the lateral lobes. (For a more detailed account of the ventricles of the teleostean inferior lobes see Goldstein ('05), pp. 189–195, figs. 13–19; Edinger ('08), fig. 171.)

(*1*) *Epithalamus.* The epithalamus of the carp is easily defined, consisting of the saccus dorsalis and epiphysis and the habenular centers, including the two habenular ganglia, the habenular decussation, or commissure and the nucleus posthabenularis, together with their connections.

The ganglia habenularum are very conspicuous in the carp, protruding for half their diameter into the median cavity (figs. 78, 81). Their cells are small and evenly distributed but thrown into groups or islands by the fibers of the tractus olfacto-habenularis and the fasciculus retroflexus (figs. 78, 81). As seen in Golgi preparations, the cells are very characteristic, of the type normal throughout the vetebrate series (fig. 75).

Nucleus posthabenularis. Immediately ventral to the habenular ganglia, the cells of the one continuous with the cells of the other, lies the nucleus posthabenularis, 'das posthabenulare Zwischenhirngebiet' of Goldstein, the 'posthabenulare Zwischenhirngegend' of Bela Haller, Meynert's nucleus of reptiles (figs. 78, 81). Rostrally, it becomes continuous with the nucleus intermedius (fig. 70), while caudally it extends beyond the level of the commissura posterior (fig. 84) always holding a position close to the median ventricle and ventral to the fasciculus retroflexus.

(*2*) *Thalamus.* At the level of the habenulae, there appear on either side, immediately ventral to the arch of the tectum, the corpora geniculata lateralia. Mesal to the lateral geniculate body lies the nucleus anterior thalami of Goldstein (figs. 78, 81). This is easily recognized, owing to its large size and its characteristic appearance, showing a ring of cells about its periphery (fig. 81).

Nucleus rotundus and associated centers. One of the most important parts of the thalamus, and at the same time one of the most difficult to understand in all its relations, is the region of the nucleus rotundus. Owing to its prominence, it has been noted by nearly every writer on the teleostean brain. It was described by Fritsch and called by him the nucleus rotundus; Bellonci used the same term, while C. L. Herrick termed it the nucleus ruber. Goldstein assigns the name nucleus ventralis thalami to this

whole region, although he shows both in his figures and descriptions that it contains different groups of cells with different characteristics. Kappers ('06) pointed out that the center previously described as nucleus rotundus is really made up of several characteristic groups of cells. That

> situated most dorsally, proximally and laterally, is the *nucleus praerotundus.* This group . . . . gradually passes backward into a much larger group situated under and lateral to the level of the nucleus rotundus and ending where the real nucleus rotundus has its largest size. This latter group, which belongs entirely to the lobi inferiores, I shall distinguish as the *nucleus subrotundus* from the *nucleus rotundus proprius,* as it extends in part under the real nucleus rotundus so that the com. horizontalis, before it enters the lower border of the latter, lies for some distance over it and between it and the nucleus rotundus proprius.

This separation of the nucleus rotundus of the earlier authors into three different components is a matter of considerable morphological importance, as will be brought out later. Kappers' description applies in a general way to the relations in the carp, with some important modifications.

At the level of the rostral margin of the lateral lobes, the nucleus prerotundus appears ventro-laterally immediately ventro-lateral to the commissura transversa (fig. 78). It consists here of a fairly compact mass of irregularly shaped cells of medium size. A short distance further caudally this nucleus lies wedged in between the lateral lobe and the commissura transversa. Dorsolaterally it forms a small protuberance on the lateral surface of the brain (fig. 81). From this point the nucleus prerotundus extends caudo-mesially to the region of the nucleus posterior tuberis. It may be compared in shape to the caudate nucleus in the human brain, with a large and conspicuous head rostrally, gradually diminishing in size caudo-mesally (figs. 84, 89, 103, 106).

The nucleus rotundus proprius is by far the largest and most conspicuous nucleus of the thalamic region. It appears rostrally at about the rostral margin of the commissura posterior and extends caudo-mesally, lateral to the nucleus prerotundus, almost to the commissura ansulata, meeting the corpus mammillare ventro-mesially (figs. 84, 89, 103, 106, 117).

Ventrally of the nucleus rotundus, extending caudo-laterally from the level of the nucleus posterior tuberis, to the level of the caudal margin of the corpus mammillare, lies the nucleus subrotundus (figs. 106, 117).

When the three components of the nucleus are considered together, it is noted that the nucleus prerotundus forms a cap over the rostro-mesal surface of the nucleus rotundus (fig. 84), decreasing in transverse diameter as the latter increases in size (fig. 89). At approximately the level where the nucleus prerotundus ends, the nucleus subrotundus is beginning to appear, embedded in the nucleus rotundus ventro-laterally (fig. 106). Further caudally (fig. 117), since the nucleus rotundus extends caudo-mesally while the nucleus subrotundus extends caudo-laterally, the two come to lie approximately in the same horizontal plane, one lateral to the other, the nucleus rotundus merging into the dorsal margin of the corpus mammillare; the nucleus subrotundus similarly ending in the nucleus cerebellaris hypothalami and losing its typical shape and appearance (see figs. 136–140, for a horizontal projection of these nuclei).

In addition to their conspicuous size, the nuclei rotundi show a characteristic structure, hardly fully brought out in any of the drawings. The nucleus prerotundus throughout most of its extent is composed of rather large scattered cells, together with small numbers of various smaller sized cells (fig. 85) showing faintly between them. Several of the cells from Golgi preparations are shown in figs. 86 to 88. The cells of the nucleus rotundus are smaller and more nearly of the same size. They are always scattered in groups or islands, giving a characteristic appearance to the nucleus (fig. 90). Figs. 91 to 94 show several from Golgi preparations. The nucleus prerotundus and rotundus combined form the 'kleinzellige' portion of the nucleus ventralis thalami of Goldstein. The most easily recognizable of these nuclei is the nucleus subrotundus, owing to its extremely characteristic appearance near its rostral end, or head. There, as shown in figs. 106 and 107, it presents a circular appearance in transection, with its cells grouped in the center and surrounded by a clear peripheral area. The cells average larger than those of the remaining two nuclei

and are noticeably so in its caudal part, where they become large, spindle shaped or pyramidal, as the nucleus cerebellaris hypothalami is approached. This nucleus corresponds to the 'grosszellige' portion of the nucleus ventralis thalami of Goldstein. By the Weigert or Ramón y Cajal methods the nucleus prerotundus and rotundus show a peculiar blotched appearance, due to the presence of small bundles of fine fibers scattered between the islands of cells; fig. 102 brings this out fairly well.

Nucleus posterior thalami. Lateral to the nucleus rotundus, at the level of the nucleus posterior tuberis, is a nucleus of very large ganglion cells, the nucleus posterior thalami, the 'Vereinsgebiet' of Bela Haller (figs. 103, 106, 117). This gradually increases in size caudally finally disappearing in the nucleus cerebellaris hypothalami. Its cells are particularly large as shown in fig. 109 from a toluidin blue preparation and figs. 110 to 113 from Golgi preparations.

Nucleus ruber tegmenti. Dorso-mesal to the caudal part of the nucleus posterior thalami, and dorsal to the nucleus subrotundus is a nucleus of extremely large cells, the nucleus ruber tegmenti of Goldstein (fig. 117).

The remaining centers of the thalami are omitted from consideration in this article as they have no special connection with the olfactory apparatus and are not necessary for purposes of orientation. This includes the *nucleus dorsalis of Goldstein*, the *nucleus corticalis of Kappers*, the *nucleus praetectalis*, and *nucleus intermedius of Goldstein.*

(3) *Hypothalamus.* The hypothalamus consists of the lobus medius and part of the lobi laterales of the inferior lobes (figs. 3, 4), together with their included centers and connections, and the hypophysis. The lobus medius consists rostrally of the tuber cinereum and caudally of the pars infundibularis. Ventro-rostrally, as previously noted, is given off the hypophysis, while extending caudally from the pars infundibularis, is found the saccus vasculosus.

Nucleus anterior tuberis. A single group of cells, the nucleus anterior tuberis, makes up the larger part of the rostral portion of the lobus medius (figs. 81, 84, 89). This is composed of small

cells, appearing as a core in the center of the nucleus (figs. 84, 89). Rostrally, the nucleus anterior tuberis is continuous past the fibrae ansulatae, Herrick's commissure, etc., into the nucleus preopticus, pars parvocellularis posterior. Caudally, the nucleus ends at the level of the lateral ventricular diverticula, leading to the lobi laterales (figs. 100,101). See also Edinger ('08), fig. 171; Goldstein ('05), text-fig. 16.

Nucleus posterior tuberis. Dorsad of the diverticula the nucleus anterior tuberis passes caudally into the nucleus posterior tuberis, immediately ventral to the tuberculum posterius (Haubenwulst) (fig. 103). This is a nucleus of small cells, similar in appearance to those of the nucleus anterior tuberis, although its cells are more evenly distributed.

Nucleus ventralis tuberis. Appearing rostrally, immediately ventral to the commissura horizontalis, is a nucleus of enormous cells, not hitherto described in the literature, which is here termed the nucleus ventralis tuberis (fig. 78). It continues for a short distance caudally, lying close underneath the median ventricle and gradually diminishing in size (figs. 81, 84).

Nucleus lateralis tuberis Laterally, appearing immediately caudal to the commissura horizontalis, at the ventro-lateral margin of the nucleus anterior tuberis, occurs a closely packed group of large cells, the nucleus lateralis tuberis (fig. 84). This is found only for a short distance at the level of attachment of the hypophysis.

Nucleus ventricularis. Close to the median ventricle, particularly as far caudal as its diverticula, may be seen a layer of densely packed cells close against the ependyma. Similar cells may be noted adjacent to the median ventricle rostrally, even before the anterior commissure. The same condition holds also for the walls of the lateral diverticula into the lobi laterales. It is noticeable that wherever these cells are found the ependyma consists of higher columnar cells than in other regions. These probably belong to the apparatus, described by Johnston, for the regulation of blood pressure in the brain.

Nucleus diffusus lobi lateralis. Throughout the peripheral portion of the lobi laterales, particularly laterally and ventrally,

is an evenly distributed area of small cells, forming the nucleus diffusus lobi lateralis of Goldstein, the substantia grisea lobi inferioris of Kappers, who divides it into a pars anterior and a pars posterior (figs. 78, 81, 84, 89, 103, 106, 117). The cells, as shown in Golgi preparations, possess elliptical or flask-shaped perikarya, with many finely spiny dendrites, resembling somewhat the undifferentiated cells of the area olfactoria lateralis. A number of the cells are shown in figs. 95 to 99. This undifferentiated area is evidently the primitive structure of the lateral lobes from which its nuclei have been gradually evolved. (Compare the condition of ganoids, according to Johnston.)

Nucleus cerebellaris hypothalami. Appearing rostrally, at approximately the middle of the longitudinal extent of the lateral lobes, occurs a nucleus of large evenly distributed, scattered cells, the nucleus cerebellaris hypothalami of Goldstein (fig. 89). This extends caudally and laterally, gradually increasing in size until it occupies a large part of the transverse diameter of each lateral lobe (figs. 89, 103, 106, 117). It extends practially to the caudal part of each lobe, laterally. A small area, under high power, is shown in fig. 108.

Corpus mammillare. The only remaining center of importance in the lobi laterales is the ganglion mammillare of Goldstein. Rostrally and dorsally it meets the tail of the nucleus rotundus; thence it extends caudally, always adjacent to the median wall of the caudal portion of each lobe (fig. 117), practically to the tip of the lobes. It is composed of very small, closely packed, evenly distributed cells of characteristic form (figs. 118, toluidin blue; 119 to 121, Golgi). Where this nucleus comes into contact with the nucleus rotundus the two may be easily distinguished by the difference in the size and arrangement of the cells.

In Weigert preparations the corpus mammillare is easily distinguished, owing to the large number of fine medullated fibers found in it, giving it a finely reticular appearance.

A number of the cell groups here introduced will not be further considered but have been mentioned in order to give an accurate understanding of the relations of the different centers.

4. THE FIBER TRACTS

a. Crural tracts

The olfactory neurones of the first order from the olfactory mucous membrane to the olfactory bulbs and their connections at that point have already been described. The connections between the bulbs and hemispheres will next be considered. It has long been known that the fibers of the olfactory tracts pass between the bulbs and olfactory lobes in two bundles; Bellonci was the first to divide the tracts into a medial and a lateral. C. L. Herrick in 1891 brings out clearly the morphological relations of these two tracts, which he calls the radix lateralis and the radix mesalis. He points out that the radix lateralis passes directly from the bulbs to the caudo-lateral part of each basal lobe, which he terms hippocampus, and that the radix mesalis decussates in the anterior commissure. Edinger ('96) figures a horizontal projection of the basal lobes of the carp, in which he traces the lateral tract, called by him the tractus bulbocorticalis, into a region termed the area olfactoria, while the median olfactory bundle, or tractus bulbo-epistriaticus, ends partly in the epistriatum of the same side, and partly decussates in the anterior commissure. Catois ('01) identifies the same two bundles as 'Le faisceau externe' and 'Le faisceau interne.' Catois is the first to point out that the medial tract consists of both centripetal and centrifugal fibers. He agrees with Edinger that it is partly crossed and partly uncrossed. Bela Haller likewise identifies the two tracts. Goldstein ('05) has worked out the relations of the bundles in more detail than his predecessors, and finds that the lateral tract, 'laterale Riechstrahlung,' originates in the lobus olfactorius anterior and ends, largely uncrossed, in the lobus olfactorius posterior, pars lateralis, while a few fibers decussate in the anterior commissure to end in the same area on the opposite side. The 'mediale Riechstrahlung' is formed, according to Goldstein, entirely from centripetal fibers, which run in several distinct bundles. The more lateral originates in the lobus olfactorius anterior, and decussates in the anterior commissure to

end in the lobus olfactorius posterior, pars lateralis, of the opposite side. The remaining two bundles originate from the formatio bulbaris; the more medial forms the commissura olfactoria interbulbaris, while the more lateral ends in the lobus olfactorius posterior, pars medialis, in which are confused the precommissural body and the epistriatum of Edinger. Kappers ('06) observes two different conditions in the teleosts examined by him. The lateral tract, or radix olfactoria lateralis, always ends in the area olfactoria posterior lateralis (area olfactoria of Edinger); in Gadus, Thynnus and Lophius it ends on the same side, however, while in Salmo it decussates in the anterior commissure to end in the opposite side. Kappers also finds that the medial tract is composed of two parts, a medial tractus olfacto-lobaris medialis and a lateral radix olfactoria medialis propria. He finds that both sets of fibers decussate and that most of them end in the area olfactoria posterior medialis, here termed epistriatum, although a few in Salmo may end in the lateral area.

In none of the previous work on these tracts in fishes have all of the connections been brought out. This is undoubtedly due, in part, to the lack of a detailed study of the olfactory bulb and in part to a failure to learn the direction of the different components by the use of the Golgi method.

The olfactory crura in the carp, as previously noted, are very long and in transections at different levels, the apparent number of tracts varies considerably. In some sections only one or two bundles will appear, while in others ten or twelve may be seen. In order to determine the number and relations of these bundles, plots were made of several complete series of serial sections of the crura, showing the number of bundles appearing in each section and their relation to one another. Micrometer measurements were used to determine the relations in all doubtful cases; that is to say, whenever in one section two bundles were found, and in the next section three, measurements were taken if there was any doubt as to which of the two gave rise to the third. In this way, it is possible to determine the number of important fiber bundles in the crura and by tracing them to their origin and termination, learn their relation to the centers of the bulbs and basal

lobes. Thus it is shown that instead of a radix medialis and a radix lateralis there are nine distinct fiber bundles running throughout the crura (figs. 123, 124, 22, 23).

(*1*) *Tractus olfactorius lateralis.* The lateral tract, the tractus olfactorius lateralis, consists of three bundles, a pars lateralis, pars intermedia and pars medialis. These are composed entirely of centripetal fibers, arising largely from mitral cells of the lateral part of each bulb. A few fibers, however, arise from stellate cells more centrally placed (fig. 124). The tractus olfactorius lateralis, pars lateralis originates, chiefly in this way, from stellate cells of the nucleus olfactorius anterior, a few of its fibers arising, however, from peripheral mitral cells (figs. 124, 137). The tractus olfactorius lateralis, pars intermedia is the largest and most important of the three. Part of its cells of origin lie in the nucleus olfactorius anterior, while the larger proportion are mitral cells from the lateral portion of the bulb rostrally and dorsally (fig. 6). One small bundle of fibers originates from the mesal part of the bulb, crossing dorsally to join the main tractus olfactorius lateralis, pars intermedia (fig. 6). The tractus olfactorius lateralis, pars medialis is small but extends throughout almost the entire length of the bulb, arising partly from mitral cells and partly from stellate cells of the nucleus olfactorius anterior (figs. 6, 124). The fibers of all three portions of the tractus olfactorius lateralis pass through the crura (figs. 22, 23), and gradually spread out above the fissura endorhinalis (figs. 24, 35) to end, without decussating, in the lateral olfactory area of the basal lobes (fig. 137), including all parts of the nucleus pyriformis and nucleus teniae. Fibers end throughout almost the entire length of the area, the fibers ending farthest rostrally arising from the tractus olfactorius lateralis, pars lateralis. All three tracts, however, give off fibers to all parts of the nucleus olfactorius lateralis, rostrally of the sulcus ypsiliformis. A larger proportion of the fibers of all three bundles end farther caudally, however, in the nucleus pyriformis, beyond the sulcus ypsiliformis, and in the nucleus teniae. Golgi preparations show that in all cases the fibers bend abruptly dorsad usually branching at their termination. The termination of the lateral tract in the

basal lobes of the carp is similar, therefore, to its ending in the majority of other teleosts; it has been possible, however, to demonstrate fibers from the tractus olfactorius lateralis in the dorsal and dorso-lateral region of the basal lobes, called by Johnston ('06) the epistriatum. This area is, therefore, simply a part of the lateral olfactory area.

(2) *Tractus olfactorius medialis.* The medial olfactory, described by the earlier workers as a single tract, and by the most recent as two, is really composed in the carp of five bundles of widely varying relationships (figs. 22, 23, 124, 136, 137).

Tractus olfactorius ascendens. The tractus olfactorius ascendens described by Kappers, in Salmo, Gadus, etc. (radix olfactoria medialis propria) as a centripetal tract is, in the carp, as shown by Golgi preparations, a centrifugal bundle, originating from cells in the nucleus medianus (figs. 27 to 31). Catois described the more medial portion of the medial tract as centrifugal, but other authors have been unanimous in considering all excepting a few commissural fibers as centripetal. The fibers of the tractus olfactorius ascendens gather from all parts of the nucleus medianus and extend rostrad to the bulb in two bundles which occupy the middle or intermediate portion of the base of each crus (figs. 24, 23, 22). On reaching the olfactory bulb the fibers gradually spread out, and end in the nucleus olfactorius anterior (figs. 124, 136).

Tractus olfactorius medialis. Medially in the bulb and crus is found the tractus olfactorius medialis. This originates almost entirely from mitral cells and contains the neurites from practically all the mitral cells far rostrally in the bulb; it may be traced much farther rostrally than any of the other tracts of the crus. Throughout most of the bulb three bundles, belonging to this tract may be identified (for two of them see fig. 6); near the caudal margin of the bulb, however, these three join to form two, which may be traced separately to their termination in the basal lobes. The two lateral bundles originate almost entirely from cells at the extreme rostral end of the bulb, joining to form the tractus olfactorius medialis, pars lateralis. This can be distinguished from the tractus olfactorius medialis, pars medialis throughout

the entire extent of the crura; for a short distance at the rostral end of the basal lobes the two are so closely joined, however, that it is difficult to identify them (figs. 24, 34). As they come into proximity to the anterior commissure they again separate, the tractus olfactorius medialis, pars lateralis holding a position dorsal to its smaller companion tract (fig. 34). From this point caudad it extends slightly laterad until the anterior commissure is reached, when it largely decussates at about the middle of the commissure, to end in the lobus pyriformis of the opposite side (figs. 35, 36, 55, 137). This agrees with the more lateral portion of the 'mediale Riechstrahlung' of Goldstein but differs from the conditions observed by Kappers, excepting for a few fibers in the brain of Salmo. A small number of fibers, however, as shown by Golgi preparations, leave the tract before its decussation to end in the nucleus preopticus (fig. 137) and the primordium hippocampi. The tractus olfactorius medialis, pars medialis originates from mitral cells of the medial surface of the bulb, and extending to the basal lobes, decussates ventral to and slightly rostral to, the tractus olfactorius medialis, pars lateralis (figs. 34, 35, 136). This forms the commissura olfactoria interbulbaris of Goldstein, the commissural fibers connecting the two olfactory bulbs, which have been described by many writers. In Weigert preparations it appears as if these fibers actually form a commissure, but when the crossing is examined in Golgi and Ramón y Cajal material, it is found that a large part of the fibers decussate in the commissure and then end almost immediately, while a few terminate at the commissure, without decussation. Many fibers terminate, also, in the pars anterior of the nucleus medianus, the pars supracommissuralis of the corpus precommissurale and possibly in the primordium hippocampi of the same side. It can not be stated with certainty that no fibers pass around to the opposite bulb; commissural fibers have, therefore, been indicated on the diagram (fig. 124). Kappers, Edinger, Bellonci and others have noted fibers belonging to the medial olfactory tract, and ending in the hypothalamus. Such an appearance is likewise common in Weigert preparations, as the fibers of the tractus olfactorius medialis, pars lateralis appear

to continue in the tractus olfacto-thalamicus medialis. Such a condition is deceptive, however, as no such fibers could be demonstrated in Golgi or Ramón y Cajal preparations. It is evident that the Weigert preparations, which fail to show the fine fibers as they approach their termination, are, therefore, unreliable in a study of the origin and termination of tracts, or the relations of two closely associated bundles.

(3) Nervus terminalis. Earlier a group of ganglion cells belonging to the nervus teminalis was described. As shown in fig. 124 the neurites of these cells pass mesad, lying for a distance between the two bundles of the olfactory nerve, along the mesal surface of the bulb. This has been demonstrated in Golgi preparations. In Weigert and vom Rath preparations, an unmedullated tract, undoubtedly formed by the central processes of these ganglion cells, extends from the same region to the hemispheres (Sheldon, '09, Sheldon and Brookover, '09). Rostrally this tract lies embedded in the tractus olfactorius medialis, pars medialis (fig. 6), on the medial aspect of the bulb. As it passes caudad throughout the crus, it still holds approximately the same position with reference to the tractus olfactorius medialis, pars medialis (figs. 22, 23). When the rostral part of the basal lobe is reached, the nervus terminalis gradually turns dorso-laterad through the tractus olfactorius medialis to lie between that and the tractus olfactorius ascendens (fig. 24). As the anterior commissure is reached, the unmedullated fibers separate from their companion tracts and decussate in the rostral part of the commissure, ending in the rostral portion of the pars commissuralis of the corpus precommissurale, as described for the nervus terminalis of selachians by Locy, and in Amphibia by Herrick (figs. 35, 136).

(4) Distribution of secondary olfactory fibers in the forebrain. It will have been noted that secondary olfactory fibers end in a very large part of the basal lobes. Fibers of the lateral olfactory tract end throughout the lateral, dorsal and latero-ventral portions of the basal lobes from the rostral end to the lobus pyriformis and nucleus teniae of the polus posterior. These fibers extend, also, into a large part of the central area formerly called striatum. The mesal tract, the tractus medialis, carries centri-

petal fibers to the nucleus medianus; nucleus supracommissuralis, nucleus preopticus and the primordium hippocampi, probably also to the nucleus commissuralis lateralis (*tr. olf. med.*); in addition to further fibers for the lobus pyriformis. The only portions of the basal lobes which do not receive secondary olfactory fibers are the nucleus entopeduncularis and, possibly, a small area in the center of the palaeostriatum. It can not be said with certainty, however, that this latter area receives no olfactory fibers of the second order; simply that such were not demonstrated.

b. The anterior commissure

The olfactory areas of the two basal lobes are connected by four sets of commissural fibers, crossing in five bundles. In the most rostral part of the anterior commissure are found numbers of fine fibers, partly medullated and partly unmedullated, bending sharply dorsad. The unmedullated fibers connect the mesal portions of the two primordia hippocampi, while the medullated join similar parts of the partes supracommissurales of the corpus precommissurale (Sheldon, '09 a, fig. 6). A short distance caudad, accompanied by unmedullated fibers, is a small commissure of medullated fibers connecting the lateral portions of the partes supracommissurales and nuclei dorsales or primordia hippocampi (figs. 35, 36). This latter bundle, as it presents points of resemblance with the commissura pallii anterior of reptiles, and the rostral portion of the commissura pallii or commissura dorsalis of Amphibia, is termed on the plates, commissura dorsalis. Morphologically, however, the fibers mentioned thus far are divisible into a commissura hippocampi, pars anterior, and a commissura corporium precommissuralium, each bundle consisting partly of each kind of fibers (fig. 138).

At the caudal part of the anterior commissure a few unmedullated fibers pass across to connect the rostral ends of the nuclei preoptici of the two lobes. This is termed the commissura nucleorum preopticorum (fig. 138).

The commissura dorsalis is closely associated with the decussation of the tractus hypothalamo-olfactorius medialis and also with

a fourth commissure, entirely unmedullated, connecting the ventral parts of the two nuclei pyriformes, and here termed the commissura hippocampi, pars posterior. Its fibers are closely intermingled with those of the decussating tractus olfactorii mediales, partes laterales, distinguishable in Weigert preparations owing to their lack of medullary sheaths (fig. 138). See also Goldstein, Taf. 11, fig. 7; Goldstein terms this the commissura olfactorii internuclearis. This commissure is shaped like a bow, with either end bent caudally to terminate in the nuclei pyriformes (figs. 36, 37, 55). This is the hippocampal commissure of C. L. Herrick, probably also the commissura interolfactoria of Kappers. This commissure offers points of resemblance with the fibers of the commissura dorsalis, which connect the two occipital poles in the frog and with a part of the commissura pallii of Kappers in the frog.

It will be noticed that the anterior commissure complex contains two bundles connected with the primordium hippocampi, and one with the nucleus pyriformis, all of which are probably represented in the commissura dorsalis, or commissura hippocampi of amphibians. The morphological significance of the regions thus connected will be considered later.

These comprise all of the connections of the basal lobes excepting those bringing them into relation with the diencephalon, together with a few praethalamic connections which will be taken up later.

c. Diencephalic connections

(1) *The tractus olfacto-habenularis.* In 1892 Edinger described for selachians a tract between the basal lobes and the ganglia habenularum which he called the tractus ganglii habenulae ad proencephalon, stating, however, the possibility that its fibers might run in the opposite direction. Such a connection was also indicated by C. L. Herrick, in the same year under the name of taenia thalami. All recent writers have observed these fibers, and have shown that they are largely ascending, from the basal lobes to the habenular ganglia of the epithalamus. Catois traces the fibers of his tractus olfacto-habenularis from the caudal

part of the hypostriatum (nucleus teniae) to the habenulae; Kappers and Goldstein make similar observations. Johnston, however ('98, '01, '02) in Acipenser and Petromyzon finds that the larger proportion of the fibers ascending to the habenulae arise from the nucleus preopticus, called by him the nucleus thaeniae ('98, '01, '02) and nucleus praeopticus ('06). Some fibers in Acipenser are traced from the nucleus postolfactorius ventralis and nucleus postolfactorius lateralis, corresponding largely to the corpus precommissurale and the area olfactoria lateralis, respectively. It will thus be noted, as Johnston himself pointed out, that the tractus olfacto-habenularis of Acipenser and Petromyzon is not the equivalent of that in teleosts, selachians, amphibians, reptiles and mammals. The conditions as observed in the carp explain this discrepancy, as in this form the tractus olfacto-habenularis is equivalent to both the tractus olfacto-habenularis of Edinger, etc., and of Johnston (figs. 140, 141, 142).

The tractus olfacto-habenularis of Catois, Edinger, Kappers, etc., the taenia thalami of Goldstein, appears conspicuously as a small, heavily medullated bundle, arising from the nucleus teniae, lateral to the fissura endorhinalis, at the level of the caudal margin of the anterior commissure. This is here termed the tractus teniae (fig. 55) and corresponds morphologically to the tractus cortico-habenularis lateralis of C. Judson Herrick in the Amphibia ('10).

It extends latero-caudad, dorsal to the bundles of the basal forebrain bundle (figs. 61, 68), where it receives a few unmedullated fibers from the nucleus intermedius, the tractus intermedio-habenularis, pars anterior (figs. 140, 141, 142), possibly homologous to the tr. septo-habenularis of Herrick. Slightly caudal to this point the tract receives a small number of unmedullated fibers from the nucleus entopeduncularis, extending dorsad from the praethalamus. This is termed the tractus entopedunculo-habenularis (fig. 72), and is probably the morphological equivalent of the lateral praethalamic portion of the taenia thalami of amphibians and reptiles. A large part of these fibers may be descending, corresponding to the tr. habenulo-thalamicus of Herrick ('10). Quite a number of fine unmedullated fibers arise

from the nucleus preopticus, pars parvocellularis anterior, to join the main tract (figs. 73, 141, 142), termed the tractus preoptico-habenularis, pars anterior. Where the nucleus intermedius becomes continuous with the nucleus posthabenularis, it gives off a few unmedullated fibers to the tractus olfacto-habenularis, the tractus intermedio-habenularis, pars posterior (figs. 141, 142). The pars magnocellularis gives rise to two sets of fibers for the habenulae, both unmedullated, a diffuse fiber connection extending dorsad, close to the median ventricle, the tractus preoptico-habenularis, pars medialis (fig. 73), and a small compact tract, which passes lateral to the basal forebrain bundle, the tractus preoptico-habenularis, pars lateralis (figs. 74, 141, 142). Further caudally tracts join the main bundle from the pars parvocellularis, pars posterior, of the nucleus preopticus, the tractus preoptico-habenularis, pars posterior; and from the nucleus posthabenularis, the tractus posthabenulo-habenularis (figs. 141, 142). Part of this may also be descending and, therefore, homologous with the tractus habenulo-thalamicus of Herrick.

All of these fibers make up the tractus olfacto-habenularis. It will be noted that the only medullated bundle is the tractus teniae; this is likewise the most conspicuous of the different fiber systems which probably explains why it is the only one previously described in teleosts. The habenular ganglia, then, receive fibers from practically all parts of the caudal portions of both the lateral and medial olfactory columns. Laterally, fibers pass up from the nucleus teniae of the lobus pyriformis, medially from the nucleus preopticus, nucleus intermedius, nucleus entopeduncularis and nucleus posthabenularis. The lateral connection is the one observed by Edinger, Catois, Kappers, Goldstein, etc., while the medial is that found chiefly in Acipenser and Petromyzon by Johnston. Apparently the largest bundle in Petromyzon corresponds with the tractus preoptico-habenularis, pars lateralis, in the carp.

Practically all of the fibers of the tractus olfacto-habenularis decussate in the commissura habenularis, the commissura superior of many writers (figs. 76, 141, 142). It is possible that a few fibers end on the same side. It is likewise possible that there are a few

commissural fibers connecting the two nuclei teniarum, taking this course, and running in the tractus teniae (Edinger ('08) fig. 231), as in the Amphibia. Such fibers would be comparable with the commissura pallii posterior (commissura aberrans) of lizards.

Several different fiber systems arising from cells in the habenular ganglia have been described. As indicated above, Edinger, in his earlier work, believed that the tractus teniae arose in the habenulae, the tractus ad proencephalon. He also describes in selachians a tract to the midbrain roof, the tractus ganglia habenulae ad mesocephalon dorsalis; a tract to the midbrain base, the tractus descendens ganglii habenulae, in addition to the long known Meynert's bundle, or fasciculus retroflexus, more recently described by Goldstein, Edinger, etc. under the name 'tractus habenulo-interpeduncularis.' Bela Haller observed fibers arising in the habenulae and entering the optic apparatus, 'Habenularwurzel des Opticus;' also a tract extending ventrad into the diencephalon, 'Hauben-Zwischenhirnbahn.'

(2) *Fasciculus retroflexus.* The fasciculus retroflexus in the carp is a strong, chiefly unmedullated tract, originating partly from cells of the habenulae (fig. 75) and partly from the nucleus posthabenularis, as pointed out earlier by Bela Haller and Goldstein (figs. 141, 142). From this point it extends caudad to the corpus interpedunculare, as described by practically all writers on the habenular connections (figs. 77, 79, 80, 82, 83, 100, 101, 102, 114, 115, 116, 122). As noted by Goldstein, it is surrounded by medullated fibers caudally. These originate from the nucleus posthabenularis and pass caudad to the commissura ansulata, which they appear to enter, turning laterad. Goldstein simply figures these fibers, giving no description of their connections.

(3) *Tractus habenulo-diencephalicus.* This tract arises in the habenulae and, descending into the more ventral diencephalic regions, is easily identified in the carp, as it is heavily myelinated. Haller traces it into the nucleus posthabenularis, while Goldstein thinks that it ends farther ventrally, possibly in his nucleus dorsalis. The tract, according to the conditions in the carp, contains both ascending and descending fibers and extends ventrocaudad from the habenular ganglia practically to the nucleus

posterior tuberis. (Tractus habenulo-diencephalicus, figs. 77, 79, 80, 82, 83, 100, 101.) Excepting its most rostral part, it is closely associated with the medial forebrain bundle dorsally, which probably accounts for the rarity with which it has been reported. Apparently most of its fibers decussate in the habenular commissure, but such could not be demonstrated with certainty.

The tractus habenulae ad prosencephalon of Goldstein, the tractus ad proencephalon of Edinger, was not identified. Of course, it is quite possible that some of the fibers of the tractus olfacto-habenularis are ascending, as Goldstein believes.

No optic connections with the habenulae could be found, as Bela Haller describes. Large numbers of cells lying in the nucleus posthabenularis, particularly near the median ventricle, give rise, however, to fibers which pass directly laterad to enter, apparently, the optic apparatus as Haller notes (figs. 76, 77, 79, 83). These require further study. Considering the intimate relation between the nucleus posthabenularis and the ganglia habenularum, an optic connection, such as Haller describes, not improbably exists in some forms.

(4) *Posthabenular-preoptic connections.* In addition to the connections already described with the fasciculus retroflexus and the optic apparatus, the nucleus posthabenularis is placed in relation with the nucleus preopticus through three sets of diffuse unmedullated fibers, a tractus preoptico-posthabenularis, pars anterior from the nucleus magnocellularis to the nucleus posthabenularis; a tractus preoptico-posthabenularis, pars posterior from the nucleus parvocellularis posterior, and the tractus posthabenulo-preopticus from the nucleus posthabenularis to the nucleus parvocellularis posterior (fig. 140).

It is evident from its position and connections that the nucleus posthabenularis is closely related with the habenulae. The two are evidently a morphological entity, the habenular ganglia developing as specialized portions of the dorsal lamina of the thalamus.

(5) *Epiphyseal fibers.* Along the caudal wall of the epiphysis runs a small medullated bundle, which extends caudad to the posterior commissure. It is possible that it gives off fibers to the

habenular ganglia as it passes them, but such could not be demonstrated with certainty.

(6) *Fasciculus medialis hemisphaerii.* This was observed first by Bellonci in Anguilla, and by him considered to be an olfactory tract of the second order from the olfactory bulbs to the nuclei rotundi. The question of the presence of such fibers in the carp has already been discussed. Edinger similarly traced a part of the fibers of the medial olfactory tract to the diencephalon, the tractus ad lobum inferiorem. C. L. Herrick identified the tract, but states that it originates in the mesaxial lobe (nucleus medianus and nucleus supracommissuralis of the corpus precommissurale), decussates as the axial commissure (anterior commissure), and then extends to the infundibulum. Herrick calls the rostral end the 'basal cerebral fasciculus,' while the diencephalic part he terms the fornix tract. Johnston ('98) describes the bundle as the tractus strio-thalamicus ventralis, passing caudad, without decussation, to end in the inferior lobes. In 1901 he points out that these fibers are largely descending, originating chiefly from the nucleus postolfactorius ventralis and to a less extent from the nucleus preopticus. It also contains ascending fibers from the corpus mammillare, most of which decussate in the anterior commissure to end in the epistriatum of the opposite side. Kappers describes the bundle in the teleosts as originating in his epistriatum (corpus precommissurale) and ending uncrossed immediately lateral to the nucleus rotundus. Goldstein gives the same origin for the fibers, but states that they decussate in the nucleus posterior tuberis. He notes also that the tract consists of more than one bundle, but fails to observe any difference in the connections of the different components.

A careful study of this tract in the carp shows that, instead of being a simple, single tract, it is really a complex of six fiber bundles each with a distinct course and connections. It likewise becomes apparent that Kappers, Goldstein, Johnston, etc., observed only a part of these components, which accounts for the differences in the course and connections of the tract as described by them.

The medial forebrain bundle first appears rostrally at the level of the anterior commissure, on either side of the mid-line. Immediately dorsal to the commissural fibers appears the tractus hypothalamo-olfactorius medialis, made up largely of fine, medullated fibers, between which are found many unmedullated in character (fig. 37). All of the fibers of this bundle are ascending, originating in the nucleus posterior tuberis (figs. 102, 104). Part of them decussate almost immediately, as shown in fig. 102, while the majority pass up on the same side to decussate in the anterior commissure, closely associated with the fibers of the commissura hippocampi, pars posterior and commissura dorsalis. Both sets of fibers terminate in the corpus precommissurale, largely in the pars supracommissuralis. This tract is that observed by Goldstein caudally, and called by him a descending tract.

Ventral to the fibers of the anterior commissure, at its level, may be seen another component of the median forebrain bundle, the tractus olfacto-thalamicus, pars ventralis (figs. 36, 37). The fibers making up this bundle appear very similar to those of the tractus hypothalamo-olfactorius medialis. They originate from the corpus precommissurale, largely in the pars supracommissuralis, and run caudo-ventrad, in a diffuse bundle, to terminate in the nucleus rotundus and the nucleus posterior thalami.

At the caudal margin of the anterior commissure a third component, the tractus olfacto-thalamicus, pars dorsalis, appears. This is a rather diffuse bundle, made up of fine medullated and intermingled unmedullated fibers, which originate largely in the supracommissural part of the precommissural body and terminate in the nucleus subrotundus. This bundle, together with the pars ventralis, was noted by Goldstein, rostrally (Taf. 11, fig. 7). He points out that one passes dorsal and one ventral to the tractus olfactorius medialis, pars lateralis, and that both originate in the medial olfactory nucleus. Apparently, however, he failed to follow all the fibers caudad, as in the more caudal region he observed only the tractus hypothalamo-olfactorius medialis, which tract he had not seen farther rostrally. The two parts of the tractus olfacto-thalamicus form the tractus olfacto-hypothalami-

cus medialis of Kappers, who failed to note the bundle from the nucleus posterior tuberis.

A short distance caudal to the anterior commissure, the medial forebrain bundle has increased largely in size (figs. 68, 69), due to the presence of a large number of short fibers, most of which are unmedullated. These are present throughout most of the extent of tract and are both ascending and descending, connecting and placing in relation the different parts of the precommissural body, nucleus preopticus and diencephalon. These fibers form the tractus olfacto-thalamicus, pars intermedia and tractus thalamo-olfactorius, pars intermedia (fig. 136).

Another factor in the increase in size of the median bundle consists in the addition to it of a few medullated fibers arising from the dorso-lateral part of the nucleus magnocellularis, forming the tractus preoptico-tuberis. These pass caudad mingled with the median forebrain bundle and end, apparently, partly in the nucleus posthabenularis, and partly in the nucleus posterior tuberis. These fibers may correspond to the 'Längsbündel' of Goldstein.

Slightly caudal to the level of the habenulae a seventh tract becomes closely associated with the median bundle, appearing to be a part of it. This is the tractus habenulo-diencephalicus of Goldstein and has already been described in connection with the habenular tracts (fig. 77).

When a careful study of the median bundle at different transsection levels is made, it is a simple matter to identify its components. Their relations rostrally have already been noted; as the tract is followed caudad it will be seen that there is a tendency for the longer components to arrange themselves in more compact bundles, with the more recently acquired fibers scattered about them (figs. 73, 74, 76). For some distance there is little change in the bundle (figs. 79, 80, 82). At the level shown in fig. 83, however, it will be noted that the fibers of the tractus olfacto-thalamicus, pars intermedia and tractus thalamo-olfactorius, pars intermedia, are decreasing in number. The remaining bundles of the complex are, at this point, separating from one another, all, however, turning ventrad (figs. 100, 101). The tractus habenulo-

diencephalicus can be traced only a short distance caudal to the level shown in fig. 101, where it ends mesal to the nucleus rotundus at the level of the nucleus posterior tuberis. The tractus hypothalamo-olfactorius medialis holds a position near the median line at this point, while the tractus olfacto-thalamici, pars dorsalis and pars ventralis are looping ventro-laterally, to pass below the nucleus rotundus (fig. 101) to their termini in the nuclei subrotundus and posterior thalami, respectively (figs. 115, 116, 122, 139).

(7) *Fasciculus lateralis hemisphaerii.* This has been known from the time of the first workers on the microscopic anatomy of the teleostean brain. It has been called by various names since the time of Stieda: pedunculus cerebri, by the earlier workers, 'basale Vorderhirnbündel' by Edinger, 'faisceau basal' by Catois, 'tractus strio-thalamicus' by Johnston, Goldstein, Kappers, etc. In practically all forms it consists almost entirely of unmedullated fibers, although it is one of the largest and most constant bundles of the brain. Earlier workers considered that it was made up exclusively of descending fibers from the cells of the corpus striatum, ending in the diencephalon. Edinger ('88) states simply that the fibers originate in the 'Stammganglion' and end in the ventral part of the 'Zwischenhirn.' He thinks it very likely that part of the fibers decussate in the anterior commissure. C. L. Herrick ('91 and '92) divides the basal forebrain bundle into two parts, both descending, a ventral peduncle arising from the rostral part of each basal lobe and ending in the caudal part of the hypoaria, and a dorsal peduncle originating in the caudal part of each lobe, and ending largely in the nucleus ruber and subthalamicus (nucleus rotundus, sensu lato). Johnston ('98) identifies three sets of fibers in the bundle, a tractus strio-thalamicus medialis, lateralis and ventralis. Johnston here includes under the name tractus strio-thalamicus "all fibers connecting the forebrain with the ventral portion of the diencephalon." His tractus strio-thalamicus ventralis is evidently a part of the medial forebrain bundle, as is also a portion of the tractus strio-thalamicus medialis, consisting of ascending fibers from the thalamus to the epistriatum, decussating in the anterior commissure. John-

ston's tractus strio-thalamicus lateralis arises from cells of the nucleus postolfactorius lateralis, while the larger part of the tractus strio-thalamicus medialis arises from the striatum proper. In 1901 Johnston modifies these descriptions somewhat. He says that the ventral bundle is composed of ascending fibers, as noted above, which end in the epistriatum of the opposite side, together with descending fibers from the nucleus preopticus. He further adds that most of the ascending fibers arise from the dorsal and lateral walls of the mammillary bodies, and run in the medial bundle. Van Gehuchten ('94) also describes ascending fibers in the tractus strio-thalamicus, stating that the bundle is made up of two kinds of fibers, those which originate in the basal ganglia and end in the inferior lobes, and vice versa. Catois observed these same two fiber groups one of which is formed by 'fibers motrices descendantes,' the other by 'fibres sensitives ascendantes.' Catois states that the descending fibers lie external and dorsal to the ascending. The descending fibers he traces largely into the nucleus rotundus, and also farther ventrally, while a few fibers extend into the basal portion of the mesencephalon. The ascending fibers are traced by Catois from the region of the infundibulum, chiefly from the more rostral part. Catois includes here the medial forebrain bundle as a part of the tractus strio-thalamicus. Kappers traces the tractus strio-thalamicus from all parts of his striatum into the pedunculi thalami, ending uncrossed partly in the nucleus rotundus, but chiefly in the nucleus subrotundus. Kappers has, however, identified a tract arising chiefly from the lateral olfactory area, the tractus olfacto-hypothalamicus lateralis, which has been included with the tractus strio-thalamicus by other authors. This passes caudad, lying immediately dorsal to the tractus strio-thalamicus, and ending after decussation in the ventral portion of the inferior lobes. Goldstein has worked out the connections of the tractus strio-thalamicus in considerable detail and finds that it originates from all parts of the striatum and that part of its fibers decussate in the anterior commissure, as Edinger suggested in 1888. Goldstein states that the crossed fibers lie mesal to the uncrossed, and that the more dorsal fibers in the praethalamic part of the tract contain

chiefly fibers from the more rostral part of the striatum. He traces strio-thalamicus fibers into the nucleus anterior thalami, nucleus dorsalis thalami, nucleus ventralis thalami, nucleus posterior thalami, nucleus anterior tuberis, nucleus lateralis tuberis, nucleus diffusus lobi lateralis. The tractus strio-thalamicus of Goldstein includes the tractus olfacto-hypothalamicus lateralis of Kappers. Johnston ('02) in Petromyzon states that the tractus strio-thalamicus is formed from the neurites of the cells of the striatum which end in the central gray of the thalamus. He also identifies fibers from the lateral olfactory centers, forming a part of his tractus olfacto-lobaris, which correspond to the tractus olfacto-hypothalamicus lateralis of Kappers.

As described here, the lateral forebrain bundle consists of the tractus strio-thalamicus, tractus thalamo-striaticus, tractus olfacto-hypothalamicus lateralis and tractus hypothalmo-olfactorius lateralis (fig. 139). Rostrally distributed through the central part of each lobe, almost at the tip of the basal lobes, may be seen in Weigert preparations many bundles of unmedullated fibers. Caudally, near the level of the anterior commissure, these bundles pass gradually ventrad, lying dorsal to the fissura endorhinalis (fig. 34). Thence these turn slightly mesad (fig. 35), constantly increasing in size through the accession of new fibers, until at the caudal level of the commissure the lateral forebrain bundle appears as a powerful tract containing many large bundles of mixed medullated and unmedullated fibers (fig. 36). As a usual thing the medullated fibers either form a sheath for the unmedullated or else form separate bundles, the two kinds of fibers being rarely intermingled in the same bundle. A large part of the fibers, as Goldstein describes, decussate in the caudo-ventral part of the anterior commissure (figs. 36, 37). Caudal to the commissure, the different bundles become more compactly arranged and extend through the pedunculi thalami close against their lateral margins (figs. 55, 61, 68).

The components of the tract, as it passes through the pedunculi thalami, are shown in fig. 139. It will be noted that the fibers are both ascending and descending and that the several bundles have somewhat different connections. In general it may be stated

that the fibers connected with the more rostral part of the basal lobes lie ventrally and medially; that those belonging to the mid-portion of each lobe hold an intermediate position, while the more caudal fibers appear dorsally in the praethalamic bundle. It will be noted, also, that those which decussate in the anterior commissure are among the more caudal fibers, while those of the extreme caudal tip of the basal lobes occupy the extreme dorsal position and form the lateral hypothalamic tracts (figs. 36, 37, 55, 61, 68, 69, 72, 73).

The lateral forebrain bundle receives from, or sends fibers to, all parts of the basal lobes excepting the corpus precommissurale, nucleus medianus, nucleus supracommissuralis, primordium hippocampi, and possibly the nucleus preopticus. The fibers from the caudal part of the lobes belong to the nucleus pyriformis chiefly, although a few fibers are undoubtedly in connection with the lateral part of the nucleus intermedius; they, therefore, form a tract corresponding to the tractus olfacto-hypothalamicus lateralis of Kappers. Kappers, however, described this as a descending tract, while it here contains both ascending and descending fibers, which reach all parts of the nucleus pyriformis (figs. 69, 72).

A large part of the fibers of the tractus strio-thalamicus, or remaining portion of the lateral forebrain bundle, are ascending and are distributed to all parts of the palaeostriatum, nucleus olfactorius lateralis, including the dorso-lateral area of the basal lobes, called epistriatum by Catois and by Johnston ('06). Many of these ascending fibers enter into relation with large association cells of these areas, their neurites enclosing the perikarya of the cells (figs. 49, 50, 51). Other ascending fibers reach the peripheral area and branch dichotomously to form tangential fibers (fig. 39), here coming into relation with the association cells and their processes. Descending fibers of the tractus strio-thalamicus arise from cells found in all parts of the same areas, palaeostriatum, nucleus olfactorius lateralis, etc., already described. The nucleus olfactorius lateralis, and most, if not all of the palaeostriatum receive secondary olfactory fibers, while the palaeostriatum receives also processes from association cells of the corpus pre-

commissurale (figs. 39, 139, *fib. precom. str.*). So far as the fiber connections are concerned, therefore, a definitely limited corpus striatum in the basal lobes can not be found, thus agreeing with the relations as shown by a cytological study.

In the pedunucli thalami, as was noted earlier, the fibers of the lateral forebrain bundle enclose the nucleus entopeduncularis, giving off collaterals to it (figs. 68, 69, 72, 139).

Throughout the extent of the pedunculi thalami little change takes place in the bundle; as it passes over the chiasma its components extend slightly ventrad, however, and now cover all of the lateral surface of the peduncles (figs. 73, 76). At the rostral margin of the lateral lobes the more ventral bundles become closely massed against the commissura transversa (figs. 77, 79), caudal to which they bifurcate, a few fibers entering the nucleus anterior tuberis (fig. 80), while many turn into the nucleus prerotundus (figs. 80, 82, 83, 100). The larger proportion of these two sets of fibers are ascending as Catois states (figs. 104, 105), although a part are certainly descending. Farther caudally the intermediate fibers of the bundle likewise turn ventro-laterad and enter the caudal part of the nucleus prerotundus, passing through it to distribute along the rostro-mesal aspect of the nucleus rotundus and to the ventral part of the nucleus diffusus lobi lateralis (fig. 83). Part of the nucleus prerotundus fibers are ascending, but a definite statement can not be made regarding those of the nucleus rotundus. In the latter case there is no doubt but that most of them are descending as C. L. Herrick, Catois and others describe, although the intermediate bundles certainly contain some ascending fibers. The fibers break up in the nucleus prerotundus and rotundus in a very characteristic manner, noted by the earlier workers on the teleostean diencephalon (see C. L. Herrick ('92), nidulus ruber). This was mentioned earlier and is shown in fig. 102. The dorsal bundles distribute caudally a few fibers to the nucleus subrotundus, nucleus posterior thalami, nucleus cerebellaris hypothalami and a large number to the nucleus diffusus lobi lateralis, ventrally and caudally. The tractus olfacto-hypothalamicus lateralis has practically the same distribution excepting that it sends no fibers to the nucleus subro-

tundus. These fibers are both ascending and descending, although most of the ascending fibers apparently arise from the nucleus diffusus. It is difficult to make positive statements regarding the cells of origin of the dorsal ascending fibers of the basal forebrain bundle owing to rather poor Golgi impregnations of adult brains in this region (fig. 139); there is no question as to their presence, however, as many such fibers can be seen leaving these bundles rostrally. In Golgi preparations of the brains of young carp fibers may be traced, nevertheless, from a nucleus apparently corresponding to the nucleus cerebellaris hypothalami into the tractus strio-thalamicus.

No strio-thalamicus fibers could be traced into the nucleus dorsalis, nucleus anterior thalami or nucleus lateralis tuberis, as Goldstein found in the forms studied by him. It is probable that the fibers which Goldstein traces into the nucleus lateralis tuberis really arise from the nucleus magnocellularis, as will be shown later. It will thus be seen that the lateral forebrain bundle contains throughout, both ascending and descending fibers connecting all of the lateral and intermediate portions of the basal lobes with practically all of the lateral and intermediate regions of the thalamus and hypothalamus, and also a part of the medial centers. It is not, therefore, the simple tract described by the earlier writers, but a complicated connection of paramount importance to the nervous mechanism.

(*8*) *The nucleus preopticus and its connections.* The fiber connections of this nucleus have been little understood by the different writers on the brains of the lower vertebrates. Johnston ('98), as noted earlier, traced fibers from it to the habenular ganglia; he also believed that secondary olfactory fibers terminate therein, although he could not demonstrate their presence. Johnston also observed fibers passing caudad, but could not trace them to their destination. In 1901 he observed fibers from it entering the tractus strio-thalamicus ventralis (tractus olfacto-thalamicus, probably pars intermedia). C. L. Herrick ('92) describes unmedullated fibers from the pars magnocellularis (nidulus praeopticus), which pass laterad into the optic tract region. Kappers notes similar fibers, which he traces ventro-

laterad, and thence caudad into the tuber cinereum, and terms the tractus praethalamo-cinereus. Goldstein traces fibers from the pars magnocellularis caudo-dorso-laterad into 'Das posthabenuläre Gebiet,' the 'Längsbündel des grosszelligen Kerns des zentralen Höhlengraues.' From the pars parvocellularis a few fibers decussate ventrally to enter the nucleus of the opposite side, the 'Commissur des kleinzelligen Kerns,' evidently the functional equivalent of the commissura anterior, pars preoptica, previously described (fig. 138). He also finds other fibers which turn caudo-lateral, dorsal to the chiasma and postoptic commissures and end among the cells of the caudal portion of the nucleus in the rostral part of the hypothalamic wall. These fibers lie lateral to the fibers from the pars magnocellularis, and mesal to the lateral forebrain bundle. Goldstein believes that they constitute "ein Längscommissur der einzelnen Abschnitte des kleinzelligen Kernes des zentralen Höhlengraues." He likewise believes that these fibers are identical with the tractus praethalamo-cinereus of Kappers and the caudal fibers of Johnston in Acipenser. Bela Haller finds a part of these fibers, but believes that they are connected with the optic apparatus.

It was pointed out earlier that in the carp there are four different habenular connections from the nucleus preopticus, corresponding partly to the connections described by Johnston in Acipenser and Petromyzon. Olfactory fibers of the second order may be traced into both the pars parvocellularis anterior and pars magnocellularis, from the tractus olfactorius medialis, pars lateralis, just before its decussation. This agrees with Johnston's conjecture (figs. 137, 139, 141). Unmedullated fibers arising from cells in the nucleus medianus and pars commissuralis of the precommissural body respectively also extend caudad, placing these two areas in relation with the different parts of the nucleus preopticus (fig. 140, *tr. med. preopt. pars ant.* and *tr. med. preopt., pars post.*). The fibers of the tractus mediano-preopticus, pars anterior pass caudad, ventral to the crossing bundles of the anterior commissure (figs. 37, 54). From it and from the tractus mediano-preopticus, pars posterior fine fibers pass ventrad to end in either

side or ventral to the recessus preopticus (figs. 54, 61). These are probably homologous with the 'Längsfasern des kleinzelligen Kerns' of Goldstein and are, perhaps, concerned with the movement of cerebro-spinal fluid.

Immediately ventral to the recessus preopticus Goldstein figures and describes a small tract (Taf. 11, fig. 7), the connections of which he was unable to identify. Fig. 62 shows a parasagittal section in which the relations of this tract are clearly shown (*tr. preopt. sup.*). It is entirely unmedullated and arises from small stellate cells (fig. 63) immediately ventral to the recessus preopticus, terminating partly in the nucleus parvocellularis posterior and partly in the nucleus magnocellularis.

The most important longitudinal caudal connection of the nucleus preopticus is the large unmedullated tractus praethalamo-cinereus. This originates largely from the nucleus magnocellularis as described by C. L. Herrick and Kappers, the fibers extending latero-ventrad (figs. 72, 73, 76). A part of the fibers, however, originate from cells of the nucleus intermedius and nucleus parvocellularis anterior (figs. 69, 72), while a few arise in the nucleus parvocellularis posterior (fig. 76). At first, the tract lies near the median line (figs. 69, 72, 73) but it gradually turns ventrolaterad (fig. 74) to lie ventral to the lateral forebrain bundle (figs. 76, 77, 79). As it is unmedullated and, therefore, of the same color as the tractus strio-thalamicus fibers, it is easily mistaken for a part of that tract and was undoubtedly so considered by the earlier authors. Immediately caudal to the postoptic commissures it bends ventro-mesad, entering the nucleus lateralis tuberis (fig. 80), where undoubtedly some of its fibers terminate, and where it probably also receives accessions. Goldstein describes and figures this tract but apparently considers it a part of the tractus strio-thalamicus, as he traces the latter tract, but not the former, into the nucleus lateralis tuberis. From this nucleus the tract extends ventrad into the nucleus ventralis tuberis (fig. 80) where it doubtless undergoes the same change as in the nucleus lateralis tuberis, thence passing on into the hypophysis, of which it forms the chief innervation, to terminate particularly in the

pars glandularis (figs. 80, 82). Kappers traces this tract, as was previously noted, only as far as the region of the nucleus lateralis tuberis, which he fails to identify.

The "Längsbündel des grosszelligen Kerns des zentralen Höhlengraues" of Goldstein could not be identified with certainty. In sagittal sections a few medullated fibers arising from the dorso-lateral cells of the nucleus magnocellularis could be observed to pass caudad, closely associated ventrally with the medial forebrain bundle as was noted earlier, apparently ending in the nucleus posterior tuberis and the nucleus posthabenularis (figs. 136, 140, *tr. preopt. tub.*). Medullated fibers extending latero-caudad as Goldstein describes were not found. It is possible, however, that the unmedullated fibers of the tractus preoptico-posthabenularis, pars anterior may correspond to Goldstein's tract.

In addition to its longitudinal and habenular connections, the nucleus preopticus possesses a number of important short transverse, or dorso-ventral connections, all of which are composed of unmedullated fibers. Rostrally there are short connections, running in both directions between the nucleus parvocellularis anterior, and both the nucleus intermedius and nucleus commissuralis lateralis, the tractus preoptico-intermedius, pars anterior; intermedio-preopticus, pars anterior; preopticus lateralis; lateralis preopticus (figs. 68, 69, 140). Further caudally are found connections between the nucleus magnocellularis and the nuclei intermedius and entopeduncularis. The nucleus intermedius connections include a double tract medially (figs. 72, 137, 140, *tr. preopt. intermed., pars med. and tr. intermed. preopt., pars med.*) and an ascending tract passing dorsad, lateral to the lateral forebrain bundle, the tractus preoptico-intermedius, pars lateralis (figs. 69, 72, 137, 140). The short entopeduncular connections are shown in figs. 69, 72, 140, *tr. preopt. entoped. and tr. entoped. preopt.* Caudally the nucleus parvocellularis posterior and the nucleus magnocellularis are related to the nucleus posthabenularis through ascending fibers to the nucleus posthabenularis from both these nuclei, and descending fibers from it to the nucleus parvocellularis posterior (fig. 140, *tr. preopt. posthab., pars ant. and pars post. and tr. posthab. preopt., pars ant.*).

There are also ascending and descending unmedullated fibers running between the nucleus posthabenularis and the nucleus intermedius (*tr. intermed. posthab. and tr. posthab. intermed.*). Connections between the nucleus entopeduncularis and the nucleus intermedius may likewise be found (*tr. intermed. entoped. and entoped. intermed.*).

All of these latter short connections contain few fibers and in many cases form little more than a reticular network between different parts of closely related regions; they can not be demonstrated by means of Weigert preparations, but come out only in the silver methods, particularly the Ramón y Cajal. They are chiefly important in emphasizing the intimate relation between all parts of the brain, and particularly, closely related morphological areas, through the formatio reticularis.

This covers all of the direct olfactory connections which could be identified, but does not include the further connections of the different tertiary thalamic centers with other points in the diencephalon, mesencephalon, cerebellum, medulla and spinal cord. Some of these are shown, however, in the Weigert transections. It is expected that an article will appear later in which the morphological relations and functions of the different diencephalic centers will be taken up in detail, in which these further connections will be brought out. Until that time, it is not deemed wise to discuss in detail the morphological bearing of the thalamic olfactory connections, although some points will be taken up later in the interpretation of results.

5. THE CONDUCTION PATHWAYS

At this point it may be well to point out the different pathways which an impulse of a given character may follow. Of the various possible, anatomically demonstrated paths open to a given impulse, the one chosen under given conditions can be unquestionably accepted only when physiological evidence can be offered in support. Nevertheless, impulses must follow conduction paths, and we may, therefore, plot out anatomically extensive impulse pathways with an exceptional degree of accuracy, as is shown in the cases where a physiological check has been used.

Descending pathways

Nervus terminalis. In this case an impulse may travel from the periphery to ganglion cells situated among the olfactory nerve fibers and thence to a decussation among the rostral cells of the bed of the anterior commissure. Its further course is not known.

The olfactory neurones of the first order end throughout the lateral, rostral and rostro-medial face of the bulb. Fibers from all three areas form the tractus olfactorii lateralis, and medialis, pars lateralis, for the nucleus olfactorius lateralis and nucleus pyriformis of the basal lobes (fig. 137). From the lobus pyriformis originate the tractus teniae for the habenula of the opposite side, and the tractus olfacto-hypothalamicus lateralis for the nucleus cerebellaris hypothalami and the nucleus diffusus lobi lateralis of the same side (fig. 137).

The corpus precommissurale stands in relation, chiefly, with the more medial portion of the bulb, through the tractus olfactorius, pars medialis and pars lateralis, which terminate largely in the nucleus medianus of the same and opposite side, in the commissure bed, and in the pars supracommissuralis of the same side. The pars lateralis, after decussation, sends also a few fibers to the nucleus intermedius (fig. 137).

From the corpus precommissurale there are, likewise, two great pathways open. In one case cells with short neurites, forming the fibrae precommissurales striatici, transfer the impulse to the palaeostriatum, whence it is carried by the tractus strio-thalamicus to the nuclei anterior tuberis, prerotundus, rotundus, subrotundus, posterior thalami, cerebellaris hypothalami and diffusus lobi lateralis of the same side; and the nuclei rotundus, subrotundus, and diffusus lobi lateralis of the opposite side (fig. 139). The other connection is through the median forebrain bundle, which places the nucleus supracommissuralis chiefly, but other parts of the corpus precommissurale as well, in relation with the nuclei rotundus, subrotundus and posterior thalami. A third connection, less prominent but of considerable morphological importance, is with the nucleus preopticus. This receives two small bundles from the nucleus medianus, the tractus mediano-

preoptici and also secondary olfactory fibers from the tractus olfactorius medialis, pars lateralis, before its decussation (fig. 136). It thus receives both secondary and tertiary olfactory fibers.

Very similar to the precommissural, and of great morphological significance, are the descending connections of the primordium hippocampi. The latter receives secondary olfactory fibers from the tractus olfactorius medialis, pars medialis, and gives rise to fibers for the tractus olfacto-thalamicus, pars dorsalis, for the diencephalon.

The important descending pathway from the nucleus preopticus is the tractus praethalamo-cinereus from the nucleus magnocellularis to the hypophysis, together with the nuclei lateralis and ventralis tuberis. Besides this there is the tractus preoptico-tuberis from the same nucleus to the region of the nucleus posterior tuberis and the nucleus posthabenularis. Both of these are probably neurones of the fourth order.

Important neurones, chiefly of the third order, connect the nucleus preopticus with the habenulae, originating from all parts of the nucleus (figs. 141, 142).

Neurones of the fourth order originate in the habenular ganglia and pass caudo-ventrad, the fasciculus retroflexus for the corpus interpedunculare, and the tractus habenulo-diencephalicus for the formatio reticularis in the region of the nucleus posterior tuberis (figs. 141, 142).

It will be noted, then, that the olfactory neurones of the first order, or olfactory nerve, carries impulses to all parts of the lateral, rostral and mesal aspects of the bulb. From the lateral part of the bulb, chiefly, but also from the mesal, impulses are carried by neurones of the second order to the lateral area of the basal lobes. Thence neurones of the third order carry the impulse either to the habenula, or else to the nucleus posterior thalami, or the diffuse cellular area of the caudal part of the inferior lobes. From the mesal portion of the bulb impulses are carried to all parts of the mesal olfactory area, or corpus precommissurale and primordium hippocampi, by neurones of the second order, which also reach the nucleus preopticus, further caudally. From the mesal area impulses may travel by neurones of the third order to the palaeostria-

tum, and thence by quaternary fibers of the tractus strio-thalamicus to practically all the nuclei of the thalamus and hypothalamus.

Or impulses will more usually take a tract of the third order, the median forebrain bundle, for the region of the nuclei rotundus, subrotundus, posterior thalami. Other impulses may continue into the nucleus preopticus with fibers of the third order, the tractus mediano-preoptici, or may reach the more rostral parts of the nucleus by means of fibers of the second order. Neurones of the third order, largely, carry impulses from all parts of the nucleus preopticus to the habenular ganglia. It is, therefore, probable that the nucleus preopticus stands in much the same relation to the habenulae as does the nucleus pyriformis. From the nucleus preopticus fibers of the fourth order reach the nucleus posterior tuberis and hypophysis, while from the habenulae such fibers pass to the corpus interpedunculare and the medial thalamus.

Motor correlation probably takes place through two connections; one of these is by means of the corpus interpedunculare, which sends fibers, according to Ramón y Cajal and Edinger, to the nucleus dorsalis tegmenti in higher forms, from which fibers undoubtedly pass into the great bulbar and spinal descending tracts for the transmission of somatic motor impulses. Other connections may also develop when this nucleus and its relations are more thoroughly worked out. Another connection is by way of the tractus thalamo-bulbares et spinales from the thalamus to the medulla and cord (Johnston, '06). In teleosts the more usual motor pathway for the simple direct olfactory impulses is probably by way of the corpus interpedunculare. This pathway is the more definitely laid down and involves the more direct connections. An impulse may pass to any part of the bulb, practically, from the olfactory mucous membrane, thence to the lateral olfactory area, thence by the definite, medullated tractus teniae to the habenular ganglia, thence by the powerful fasciculus retroflexus to the corpus interpedunculare and thence to the tegmental region of the mesencephalon, whence it may come into relation with the motor areas of the midbrain, medulla and spinal cord.

The olfactory connection with the thalamus is not so simple and direct. An impulse must pass from the corpus precommis-

surale by way of the comparatively few fibrae precommissurales striatici to the palaeostriatum and thence through the tractus strio-thalamicus, or else from the corpus precommissurale by way of the descending fibers of the medial forebrain bundle. In neither of these cases do we find so definite and compact a pathway as that first outlined, wherefore we may conclude that the first is the more usual path for the direct olfacto-motor reflexes. Another possible motor connection is through the preoptico-habenularis fibers to the habenular region, and thence through the fasciculus retroflexus, as above indicated. This is probably a very unusual pathway as the connections just mentioned are very diffuse and are undoubtedly simply the vestiges of a once powerful pathway, now of less functional importance (cf. Acipenser). The functions of these latter pathways will be considered later.

It is quite probable that there exist also somatic fibers connecting the epithalamic with the visual centers, although such were not demonstrated (Herrick, '10b, pp. 468–469). The relation between the ventral hypothalamic region and the visceral (gustatory) pathways in teleostean fishes will be brought out later (see also the discussion in the above mentioned paper of Herrick).

Ascending pathways

There is no evidence for the existence of centrifugal fibers in the olfactory nerve bundles. Ascending fibers from the diencephalon include fibers from the lateral and ventral portions of the inferior lobes to the nucleus pyriformis (tractus hypothalamo-olfactorius lateralis); fibers from the ventro-lateral part of the inferior lobes, the nucleus prerotundus and nucleus anterior tuberis especially, and possibly the nucleus rotundus to the palaeostriatum and nucleus olfactorius lateralis by way of the tractus thalamo-striaticus; and the fibers from the nucleus posterior tuberis to the corpus precommissurale. From the corpus precommissurale, nucleus medianus, fibers pass to the nucleus olfactorius anterior in the tractus olfactorius ascendens (figs. 136, 137).

C. Judson Herrick traces the gustatory fibers of the fourth order into the caudal portion of the inferior lobes; it is likewise probable that tactile and other general sensory fibers reach the dorsal thalamic region through the medial lemniscus fibers. It is, therefore, probable, as Johnston and Herrick have already pointed out, that the ascending fibers from the pars dorsalis of the thalamus and from the hypothalamus are in the nature of general somatic and visceral sensory forebrain connections, respectively. The relations of the nucleus posterior tuberis need to be better understood, however, before the function of this ascending tract can be stated positively. It may be a connection for the transmission of visceral and somatic sensory impulses to the olfactory bulbs through the tractus olfactorius ascendens.

Association connections

Cajal and Golgi preparations show that practically all parts of the brain are permeated by a closely meshed reticulum of fine fibers, the 'Punktsubstanz' or formatio reticularis. In certain preparations it is almost impossible to identify individual cells, so close is the fibrous mesh. All parts of closely related regions, such as the different nuclei of the corpus precommissurale, are also placed in relation by means of large numbers of short connections. The same holds true with respect to regions derived from the same morphological structure. This explains the connections between the nucleus intermedius and the nucleus posthabenularis, both of which are probably parts of the same dorsal olfactory column. It was noted earlier how the nucleus medianus separates into dorsal and ventral columns; how the dorsal continues caudo-laterad as the nucleus supracommissuralis, nucleus intermedius, nucleus posthabenularis and habenulae; and how the ventral continues as the pars commissuralis, nucleus medianus and the nucleus preopticus. It is, therefore, to be expected, after what has been said regarding the close connection of associated regions that these two dorsal and ventral columns would possess short association connections. Such is the case and, while these fibers have been given the name of tracts, they are really all a

part of the same set of association fibers. The connections include all the nucleus preopticus-nucleus intermedius, posthabenularis, ganglia habenularum, nucleus commissuralis, nucleus entopeduncularis connections (fig. 140). Such connections also exist between the corpus precommissurale and the primordium hippocampi.

Commissural connections

These include the commissura interbulbaris between the two olfactory bulbs (?); the commissura hippocampi, pars anterior connecting the two primordia hippocampi or nuclei dorsales; the commissura hippocampi, pars posterior, joining the lobi pyriformes; the commissura corporium precommissuralium, between the partes supracommissurales; and the commissurae nucleorum preopticorum, all present in the anterior commissure. The pyriform lobes may also be connected through the superior or habenular commissure forming a commissura aberrans.

The formatio reticularis

In any discussion of the different pathways it must never be forgotten that the fine, reticular network of the formatio reticularis type is of great functional importance. In the past the tendency has been to consider only the tracts laid down in definite bundles. It is probable that in the phylogeny of a fiber tract the heavily myelinated bundle is the latest stage. In early stages, different areas are connected by a diffuse network of unmedullated fibers, through which impulses may take many courses. As phylogenetic development proceeds, impulses tend to take more and more definite paths through the maze of the reticulum; thus the diffuse unmedullated fiber connection is formed. Next this diffuse connection becomes more compact and usually myelinated. It should not be implied that the myelinated tract is more efficient for all connections, as it probably comes into existence chiefly when there is necessity for a stereotyped reflex; to prevent, possibly, 'loss of current' through diffusion, to use an electrical analogy. In spite of this, there is no question but that the more diffuse connections are of the utmost importance in putting into

relation different parts of the nervous system, and in causing it to react as one correlated, organic whole.

6. THE MORPHOLOGICAL AREAS OF THE FOREBRAIN

On the basis of the facts brought forward in the previous discussion, the forebrain of teleosts may be divided into morphologically distinct centers, according to the following table:

- Telencephalon.
    - Bulbus olfactorius
    - Nucleus olfactorius anterior
    - Pars lateralis hemisphaerii (pars dorso-lateralis, Herrick)
        - Nucleus olfactorius lateralis
            - Tuberculum anterius
            - Tuberculum laterale
        - Lobus pyriformis
            - Nucleus teniae
    - Pars medialis hemisphaerii (pars ventro-medialis, Herrick)
        - Corpus precommissurale
            - Nucleus medianus
            - Pars commissuralis
            - Pars supracommissuralis
            - (Nucleus intermedius, in part, at least)
    - Primordium hippocampi, or nucleus olfactorius dorsalis (pars dorso-medialis, Herrick)
    - Palaeostriatum (pars ventro-lateralis, Herrick)
        - Nucleus commissuralis lateralis
        - Nucleus entopeduncularis
    - Nucleus preopticus
        - Pars parvocellularis
        - Pars magnocellularis

Johnston ('11) has made an important contribution to the morphology of the forebrain of fishes in his analysis of the 'somatic area' of selachians. This paper came into my hands after the present contribution was ready for the press, and I have not had an opportunity to make a thorough inquiry into the teleostean homologies of this selachian area. Pending further study of this question, I may say that it now seems probable that some or all of the following regions of the carp brain correspond with the selachian somatic area of Johnston: palaeostriatum, nucleus teniae, nucleus intermedius of the precommissural body, nucleus

commissuralis lateralis and nucleus entopeduncularis. The fiber connections of several of these nuclei are still very imperfectly known and their morphological interpretation should therefore be considered purely provisional until this knowledge is extended.[1]

III. DISCUSSION

The structural plan of the teleostean diencephalon and telencephalon is very different from that of any other vertebrate type excepting the higher ganoids (notably Amia); but as we follow down the phylogenetic series through the lower ganoids to the generalized fishes, we approach progressively nearer to the common vertebrate type. When the development of the teleostean brain is more fully known it will probably prove easy to follow here also the sequence of form changes from a generalized type.

It is generally accepted that the primitive form of the vertebrate central nervous system was a simple epithelial tube and that from its rostral end two pairs of lateral vesicles were evaginated. One of these comes from the diencephalon to form the optic vesicles: the other comes from the telencephalon to form the cerebral hemispheres. The telencephalon must be defined, as taught by His and Johnston, as the rostral segment of the neural tube, including the hemispheres evaginated from it, and not as the hemisperes alone, as in the BNA tables.

There is the greatest diversity in different vertebrate types in the relative amounts of the telencephalic segment which are evaginated into the hemispheres, but in no case is the whole of this segment represented in the hemispheres. Accordingly, we subdivide the telencephalon into telencephalon medium and

[1] Johnston's still more recent paper on the telencephalon of ganoids and teleosts (Jour. Comp. Neur., vol. 21, no. 6, December, 1911), appeared while this contribution was in press. His results differ in some matters of fact and in several matters of interpretation from my own. So far as these concern the somatic or non-olfactory connections, they do not fall within the scope of this article. Some of his morphological conclusions I think rest upon an incomplete knowledge of the anatomical facts; but since the homologies of the telencephalic and diencephalic centers in the carp and other lower vertebrates will be fully discussed in a forthcoming paper, Johnston's conclusions will not be further considered at this time.

cerebral hemispheres, and recognize that in general the hemispheres increase at the expense of the telencephalon medium as we ascend the phylogenetic series. For further discussion of this question, see Johnston ('09) and Herrick ('10 b). The latter author, on the basis of the examination of a series of embryonic and adult brains of different vertebrates, has studied the method of evagination of the cerebral hemispheres in relation with the functional connections of the different parts of the neural tube involved in this process and has devised a schematic picture of the probable relations of the functional subdivisions of the neural tube in a primordial vertebrate whose optic and cerebral vesicles were still in the unevaginated condition ('10 b, fig. 72). See also Johnston ('11), fig. 82.

In such an ancestral type the sulcus limitans, terminating in the preoptic recess, separates the ventral lamina of the neural tube (Bodenplatte or hypencephalic region of His) from the dorsal lamina (Flügelplatte or epencephalic region). The ventral lamina therefore, ends in the chiasma ridge and all of the diencephalon and telencephalon dorsal and rostral to the sulcus limitans belongs in the primary dorsal lamina, i.e., to the sensory or receptive region. The chief sensory function of this region was, in the telencephalon, primitively, olfaction. The tissue in the ventral part of this region, which lies in contact with the ventral (efferent) lamina behind, secondarily assumed the function of motor correlation tissue, this part being usually above fishes separated from the dorsal part by a sulcus, the sulcus medius (sulcus Monroi of authors), which in higher forms extends caudad from the interventricular foramen. By a process of further differentiation the part above the sulcus medius becomes divided into epithalamus and pars dorsalis thalami, and the part below the sulcus medius into pars ventralis thalami and hypothalamus, the latter extending forward beyond the chiasma ridge into direct continuity with the preoptic nucleus.

The relations just described are preserved in the diencephalon of adult brains of many of the Ichthyopsida and are visible in embryos of many higher vertebrates. A transection taken through the rostral end of the diencephalon, accordingly, in these

lower vertebrates shows, in addition to the membranous median plates in the roof and floor, four longitudinal columns or laminae on each side, viz., the epithalamus, pars dorsalis thalami, pars ventralis thalami and hypothalamus (fig. 128). The last two contain motor correlation tissue, with somatic and visceral elements, respectively, predominating.

In the primordial vertebrate these four columns probably extended forward into the telencephalon without fundamental change. In all existing vertebrate types variable amounts of this telencephalic tissue are evaginated to form the cerebral hemispheres. The olfactory bulb clearly formed the initial center of evagination. In cyclostomes the hemisphere is composed of olfactory bulb, with part of the secondary olfactory nucleus (these coming from the telencephalic extension of the pars dorsalis thalami), and a very small corpus striatum, this being an extension of the pars ventralis thalami. In the lower elasmobranchs the olfactory bulb is fully evaginated and the telencephalon medium greatly elongated, with great thickening and a very slight evagination of its rostral end. In the higher sharks the telencephalon medium is shortened in correlation with an increase in the thickening of the tissue about the lamina terminalis and the further evagination in this region of the secondary olfactory centers.

The Dipnoi show a very different line of specialization. The olfactory bulbs are in all cases fully evaginated. The telencephalon is not greatly elongated (except in adult Ceratodus) and its lateral walls are uniformly thickened and more or less completely evaginated to form the cerebral hemispheres, whose form and structure, especially in the case of Lepidosiren, are very close to those of Amphibia.

The morphology of the amphibian cerebral hemisphere has been fully discussed in the paper cited (Herrick, '10 b), the author showing that it is naturally divided into four parts (exclusive of the olfactory bulb), viz., (1) pars dorso-medialis (primordium hippocampi), (2) pars dorso-lateralis (primordium of the pyriform lobe), (3) pars ventro-lateralis (primordium of the corpus striatum) and (4) pars ventro-medialis (precommissural body and septum). He shows further that these four parts are the telen-

cephalic extensions respectively of (1) the epithalamus, (2) the pars dorsalis thalami, (3) the pars ventralis thalami and (4) the hypothalamus and tissues surrounding the preoptic recess. The cerebral hemispheres of amniote vertebrates are modifications of this fundamental pattern.

The teleostean forebrain conforms neither to the selachian nor to the dipnoan and amphibian type. Further analysis of the series of ganoidean types and of the ontogeny of the teleosts will doubtless shed light upon the steps by which the teleostean pecularities have been acquired. The study of the form and fiber connections of the adult brain, together with the available data bearing on its phylogeny and ontogeny, suggests the following interpretation.

It is evident that the teleostean olfactory bulbs are completely evaginated and that they have carried out with them a small amount of secondary olfactory tissue, the nucleus olfactorius anterior. The remainder of the telencephalon remains unevaginated as the telencephalon medium, which is, moreover, considerably elongated. The failure of any considerable part of the telencephalon, except the olfactory bulbs, to evaginate laterally is the basis of its difference from that of the Dipnoi and Amphibia. The fact that the increase in its tissue takes place uniformly throughout its length or somewhat more at its caudal end instead of at its rostral end is the basis of its difference from the elasmobranchs.

The increase in the mass of the telencephalon occurs under the influence of two chief factors: (1) olfactory impulses coming in by way of the olfactory bulbs, (2) non-olfactory sensory impulses coming in for correlation purposes from the thalamus and hypothalamus. The correlation sought in the lower forms was exclusively with the olfactory apparatus; olfacto-somatic in the case of the thalamic tracts, and olfacto-visceral in the case of the hypothalamic tracts. In higher vertebrates the non-olfactory systems effect correlations *inter-se* thus giving rise to the neopallium; but little, if any of this sort of correlation occurs in fishes.

In the teleostean brain, as has been pointed out earlier, the arrangement of the telo-diencephalic centers in the form of longitudinal columns, is plainly evident. At the rostral end of

the basal lobe the ventro-medial column appears in its primitive relations, forming here the nucleus medianus of the precommissural body. Passing caudad the nucleus medianus bifurcates at the anterior commissure into the dorsal pars supracommissuralis and the ventral pars commissuralis or commissure bed. The latter is directly continuous with the nucleus preopticus, which in turn grades almost insensibly into the hypothalamic nuclei. The pars supracommissuralis becomes continuous caudally with the nucleus intermedius. The cells of the latter nucleus likewise grade over into those of the nucleus posthabenularis and habenula, but this connection is probably secondary, as will be brought out later.

The other diencephalic columns are interrupted at the level of the velum transversum save for the fiber tracts of the basal forebrain bundles. Dorsal to the ventro-medial column lies the primordium hippocampi rostrally, immediately above the corpus precommissurale. This, the dorso-medial column of Herrick, is probably the telencephalic extension of the epithalamic habenula and nucleus posthabenularis of the diencephalon.

The nucleus entopeduncularis probably belongs to the same column as the pars ventralis thalami, the pars ventro-lateralis of Herrick, which expands rostrally to form the palaeostriatum. In the evaginated hemispheres of the Dipnoi and Amphibia the striatal complex is carried outward into the ventro-lateral wall of the hemisphere vesicle. In teleosts the wall as a whole does not evaginate in this way; but the striatum complex, with the associated lateral forebrain tract, moves outward within the solid basal lobe away from the ventricular surface and toward the lateral surface of the brain, a movement which has been carried to a greater extreme in the 'somatic area' of elasmobranchs (Johnston, '11). The precommissural body and the palaeostriatum are to be regarded as extensions of the hypothalamus and ventral part of the thalamus respectively and, therefore, as equivalent to the pars basalis, or pars subpallialis, of the amphibian hemisphere. The remainder of the basal lobe is the extension of the epithalamus and dorsal part of the thalamus and, therefore, is the equivalent of the pars pallialis of the amphibian brain.

The olfactory crus is attached to the rostral end of the basal lobe by two systems of tracts, a medial and a lateral. The former, as in Amphibia, connects chiefly with the precommissural body (tractus olfactorius medialis) and in smaller measure with the dorso-medial part of the basal lobes termed primordium hippocampi in this paper, this relation being in principle similar to that of Amphibia and higher forms. The closely associated nervus terminalis and tractus olfactorius ascendens have been discussed in another connection. The tractus olfactorius lateralis connects chiefly with the lateral part of the basal lobe, the nucleus olfactorius lateralis and the nucleus pyriformis. These nuclei correspond in a general way with the dorso-lateral part of the amphibian hemisphere, or primordium of the lobus pyriformis. Like the palaeostriatum, they tend to move laterad away from the ventricular and toward the lateral surface of the basal lobe.

In vertebrates with evaginated hemipheres the two dorsal parts (pars pallialis) lie on opposite sides of the lateral ventricle and in later phylogenetic stages become respectively the hippocampus and the pyriform lobe. In the teleosts these parts are very imperfectly separated, especially at the rostral end of the basal lobe; here both are parts of a common secondary olfactory nucleus. Incident to the progressive enlargement of the telencephalon without the evagination of its walls, the thickened secondary olfactory nucleus moves laterad, carrying with it the taenia, or line of attachment of the membranous roof, which accordingly becomes dilated laterally. (See figs. 126 to 134 illustrating the arrangement of these parts and the process of eversion.)

It will be observed that the teleostean form has not been reached by a simple process of eversion of the whole wall such as that suggested by Mrs. Gage ('92; see fig. 135); for that would bring the primordium hippocampi, which borders the taenia in Amphibia, far ventro-laterally in the teleosts. This appears not to be the case, but a portion of the dorsal secondary olfactory nucleus retains its dorso-medial position with reference to the other massive structures, in spite of the lateral movement of the taenia. The movement in question is not, in fact, a simple lateral bending of the whole wall at the sulcus limitans telencephali,

but rather a gradual plastic movement of the material, such that, while the precommissural body and the medial part of the dorsal olfactory nuclei remain in the original position, the intervening portions of the lateral wall move toward the lateral part, thus bringing the dorsal olfactory nucleus and the precommissural body into contact at the sulcus limitans. The palaeostriatum moves laterad only a short distance, coming to occupy the middle of the basal lobe. But a portion of the dorsal olfactory nucleus and the whole of the lateral nucleus move to the extreme ventro-lateral margin, carrying the taenia with them, thus forming at the rostral end of the basal lobe the tuberculum laterale, and at the caudal end the nucleus pyriformis.

The tuberculum anterius, tuberculum laterale, and nucleus dorsalis are parts of the undifferentiated secondary olfactory nucleus. The precommissural body and pyriform nucleus are more highly differentiated parts of the secondary olfactory nucleus which have developed under the influence of ascending fibers of the medial and lateral forebrain tracts respectively. The palaeostriatum has become an efferent correlation center relatively free from direct olfactory connections. It is interesting to note that the termination of the lateral hypothalamic tract caudally in the teleosts has brought about the development of the nucleus pyriformis at that point, while in the selachians the more rostral ending of this connection (tractus pallii) has induced the formation of the nucleus olfactorius lateralis and primordium hippocampi in a correspondingly different position.

The selachians exhibit a considerably more highly differentiated condition of all of the forebrain centers than is found in the teleosts (cf. Johnston, '11). The selachian ascending tract from the hypothalamus to the primordium hippocampi (tractus pallii), in teleosts is probably represented in the tractus hypothalamo-olfactorius lateralis, a condition which resembles that of amphibians (Herrick, '10, p. 444).

The nucleus olfactorius dorsalis or primordium hippocampi receives some fibers from the tractus olfactorius medialis, and this connection is probably the reason why this portion of the undifferentiated secondary olfactory nucleus retains its dorso-medial

position during the lateral eversion of the remainder of this nucleus. The adult configuration is such as to suggest that the nucleus dorsalis is homologous with the amphibian primordium hippocampi and the sulcus limitans telencephali with the fissura limitans hippocampi of Herrick (fissura arcuata of Gaupp). The latter homology is however, manifestly incomplete, for the fissura limitans hippocampi is a total fissure involving the whole wall of an evaginated hemisphere, while the teleostean sulcus limitans is an ependymal groove within the ventricle of the telencephalon medium. The two sulci in question separate homologous parts of the brain and are as nearly homologous as the topographic relations of these two types of telencephalon permit.

Some justification may be found for the homology of the nucleus olfactorius dorsalis with the primordium hippocampi of Amphibia, although the apparent resemblance in position is an argument rather against it than for it. It must not be forgotten that the nucleus dorsalis occupies a dorso-median position *below* the telencephalic ventricle, not above it, as in Amphibia. In the process of eversion, to which reference was made above, the whole of the dorsal nucleus might be expected to follow the taenia in its lateral movement. The fact that a part of this nucleus retains its position at the dorso-medial border of the basal lobe has been already explained as due to its connection with the tractus olfactorius dorso-medialis. This is a primary connection of the primordium hippocampi; cf. fig. 125 with C. J. Herrick ('10 b), figs. 72, 73, 83 and 84, the nucleus olfactorius dorsalis or primordium hippocampi of the teleost being the functional equivalent of Herrick's dorso-medial ridge in spite of its position far removed from the taenia. Nevertheless, the nucleus dorsalis shows few other resemblances with the primordium hippocampi. It has not been shown to receive large numbers of olfactory fibers of the third or higher orders; it sends very few fibers to the anterior commissure complex to form a commissura hippocampi and no clearly defined columna fornicis fibers appear to arise from it, though possibly the medial forebrain bundle may contain fibers of this type.

It is concluded, therefore, that the materials found in the amphibian primordium hippocampi are not completely separated in the teleosts from the other elements of the secondary olfactory nucleus, being represented chiefly in the nucleus olfactorius dorsalis or primordium hippocampi and to a less degree perhaps in the nucleus olfactorius lateralis and nucleus pyriformis.

The term 'epistriatum' has not been used in this article in the description of the telencephalic nuclei, owing to the fact that it has been applied by different authors, with resulting confusion, to morphologically different structures. It was originally used by Edinger ('96), to designate a structure found dorsal to the striatum in the lateral wall of the reptilian forebrain. Its connections here show clearly that it is morphologically a lateral structure, corresponding to the nucleus sphaericus of students of reptiles. The epistriatum of birds, as described by Edinger, is likewise a lateral structure. Turning to the so-called epistriatum of the anamniotes, a different condition is immediately noted. Edinger ('06a) and Kappers ('06) describes as epistriatum in teleosts a medial area reached by the tractus olfactorius medialis which seems to include a part of our precommissural body, but in their later works this name is applied to our nucleus olfactorius dorsalis. Catois uses the term for the dorsal portion of the palaeostriatum. Johnston ('06) places the epistriatum of teleosts on both the medial and lateral parts of each basal lobe, although these two areas belong to morphologically different structures. It is difficult to see how the term can continue in use without constantly increasing confusion. Even if all workers had clearly in mind the morphological characteristics of the different varieties of epistriatum, it would seem unwise to use the same name, even with a modifying adjective, as does Kappers in his later work, for such morphologically different structures.

From the preceding discussion it is clear that the localization of function in the telencephalon of teleosts has not advanced so far as in Amphibia and Dipnoi with more fully evaginated hemispheres. This is probably the explanation of the fact that the diencephalic regions are also far less clearly analyzed than in Amphibia, and that nearly all parts of the basal lobes seem to be

connected with both hypothalamic and thalamic centers. But the discussion of these relations can be taken up more profitably after the connections of the diencephalic nuclei are more fully analyzed and particularly, after their embryological development has been studied.

Some comment should be made on the bearing which the data given in this article make with respect to the morphology of the forebrain tela. It is clear from the facts presented that the forebrain of the teleostean fishes contains primordial pallium and also the primordium of all important morphological structures found in the forebrain of higher vertebrates. The pallium of Rabl-Rückhard, then, is not the morphological equivalent of any portion of the wall of the forebrain of higher vertebrates but is simply a tela, derived from the Deckplatte of His. In fact there is no evidence anywhere in the phylogeny of the vertebrate brain that the Deckplatte gives rise to a nervous structure. The evidence which has been offered, then, gives additional support to the views of Studnička, already accepted by Kappers, Johnston, Edinger and Herrick.

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EXPLANATION OF FIGURES

All drawings are made from the brain of the carp, Cyprinus carpio L. The individual specimens from which these are made range from 15 to 30 cm. in length for the Golgi preparations; 25 to 40 cm. for those prepared with toluidin blue and the method of Ramón y Cajal, and 35 to 60 cm. for the Weigert preparations. Figs. 1 to 4 were drawn with the use of a dissecting microscope; for all others there were used a camera lucida and Zeiss microscope with the following objective and ocular combinations: compensating ocular 4*, objective A*; compensating ocular 8, objective A*; ocular 2, objective AA; ocular 4, objective AA; ocular 6, objective AA; compensating ocular 6, objective AA; compensating ocular 4, apochromatic objective 16 mm.; compensating ocular 4*, apochromatic objective 8 mm.; compensating ocular 18, apochromatic objective 4 mm.

On all figures from longitudinal sections an arrow (⟶) is placed always pointing rostrad. Where a double pointed arrow (⟵⟶) appears after the name of a tract it signifies that the tract in question contains both ascending and descending fibers; the name used on the figures is, however, always that of the descending tract. All figures from the Weigert or toluidin blue method are from transections; in the case of the latter every cell appearing in the section is drawn in with a camera lucida in order to obtain the proper grouping.

The eight diagrams, figs. 125, and 136 to 142, consist in each case of a basal diagram, the same in figs. 125 and 141, and in figs. 136 to 140, 142; to which is added in one or more different colors, the fiber connections. The two different basal diagrams are made from series of adjacent sections by the Weigert method, sagittal in the case of figs. 125 and 141, frontal in figs. 136 to 140, 142. These are drawn with the aid of a camera lucida, a Zeiss comp. oc. 4*, and objective A*, and are superimposed in such a way as to bring as many as possible of the structures to be considered into one figure. The relations are not, of course, accurate for any one given plane. The fiber tracts are represented by simple lines showing the course of each tract and its connections. The tracts so represented, are not, of course, equal in respect to number of fibers; some, such as the lateral forebrain bundle, are composed of an enormous number, while others, such as the tr. preoptico-habenularis, pars posterior, contain only a few.

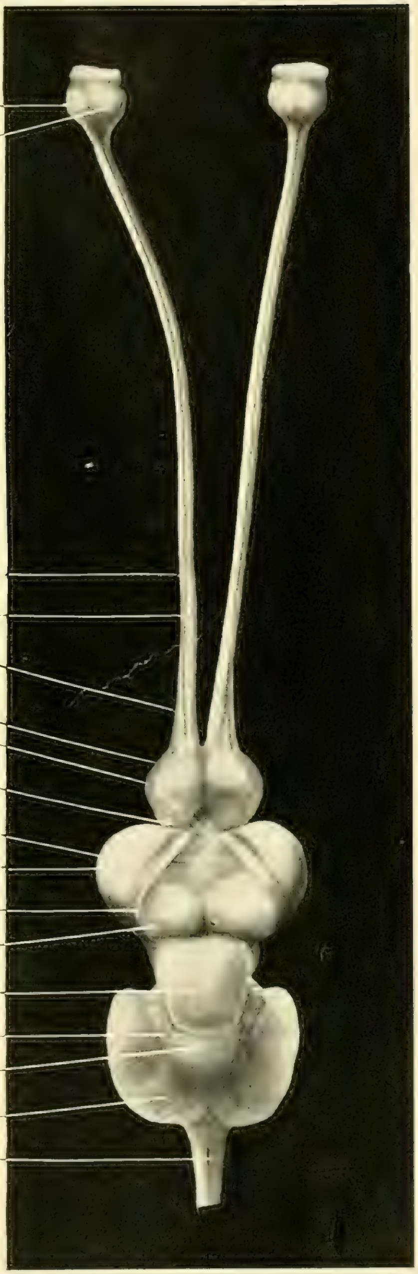
PLATE 1

EXPLANATION OF FIGURE

1 Dorsal aspect of the brain of the carp. × 2.

a, dorsal-lateral protuberance on the surface of the olfactory bulb caused by the entering fibers of the olfactory nerve; *bulb. olf.*, bulbus olfactorius; *cbl.*, cerebellum; *crus olf.*, crus olfactorium; *gang. bas.*, ganglion basale of the cerebral hemispheres; *lob. fac.*, lobus facialis or tuberculum impar; *lob. vag.*, lobus vagi; *mesotela*, membranous roof of the mesencephalon; *rhinotela*, membranous roof of the cavity of the olfactory crus, the *rhinocele; sac. dors.*, saccus dorsalis, enclosing the corpus pineale; *sp. cord.* spinal cord; *tela.*, membranous roof of the fourth ventricle; *tectum*, tectum mesencephali; *torus long.*, torus longitudinalis; *valvula*, valvula cerebelli, showing through the membranous mesotela.

PLATE 1



1

KATHARINE HILL, DEL.

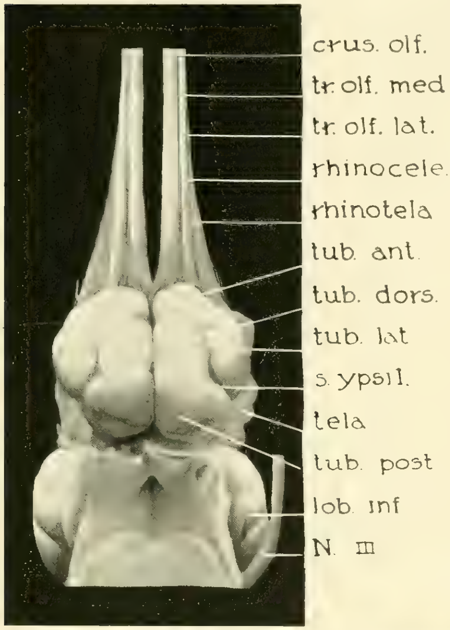
PLATE 2

EXPLANATION OF FIGURES

2 Dorsal aspect of the rostral end of the brain. × 4. The optic lobes are removed and the tela of the cerebral hemispheres, the so-called pallium, is torn from the dorsal surface, exposing the basal ganglia.

3 Left lateral aspect of the rostral end of the brain. × 4. Optic lobes and tela as in fig. 2.

crus olf., crus olfactorium; *hyp., pars gl.*, hypophysis, pars glandularis; *hyp., pars. nerv.*, hypophysis, pars nervosa; *lob. inf.*, lobus inferior or hypoarium; *N. III*, nervus oculomotorius; *n. opt.*, nervus opticus; *n. cbl.*, protuberance caused by the development of the nucleus cerebellaris hypothalami of Goldstein; *n. prerot. + n. rot.*, protuberance caused by the development of the nucleus prerotundus and nucleus rotundus centrally; *s. ypsil.*, sulcus ypsiliformis of Goldstein, the rostral prolongation of which corresponds morphologically to the fovea endorhinalis interna of Kappers and Theunissen ('08); *tela*, this indicates the torn edge of the tela or pallium which covers the basal ganglia, extending rostrally over the olfactory tracts to the olfactory bulbs, and caudally between the two halves of the tectum, over the valvula; *tr. olf. lat.*, tractus olfactorius lateralis, the radix olfactoria lateralis of Kappers; *tr. olf. med.*, tractus olfactorius medialis, including also the tractus olfactorius ascendens and nervus terminalis; the corresponding tracts, according to Kappers are, tractus olfacto-lobaris medialis and radix olfactoria medialis propria; he failed to note the nervus terminalis; *tub. ant.*, tuberculum anterius; due chiefly, to the presence underneath, of the rostral end of the nucleus olfactorius lateralis ; *tub. dors.*, tuberculum dorsale, enlargement due to the development of the nucleus olfactorius dorsalis; *tub. lat.*, tuberculum laterale, caused by the development of the nucleus olfactorius lateralis; *tub. post.*, tuberculum posterius, due to the development of the nucleus pyriformis.



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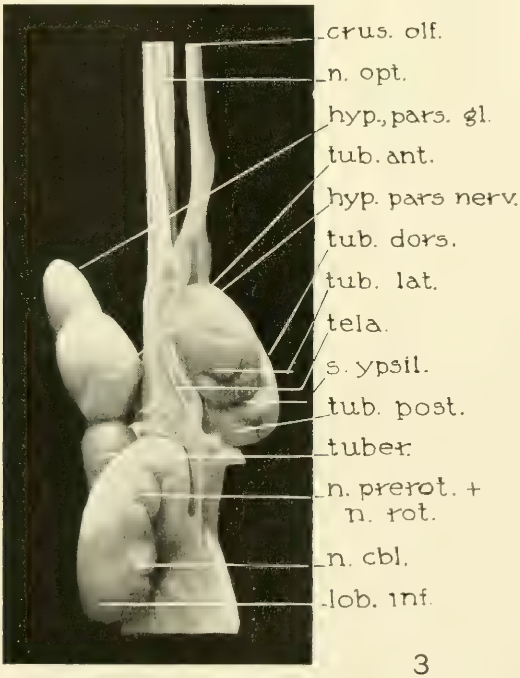


3

KATHARINE HILL, DEL.

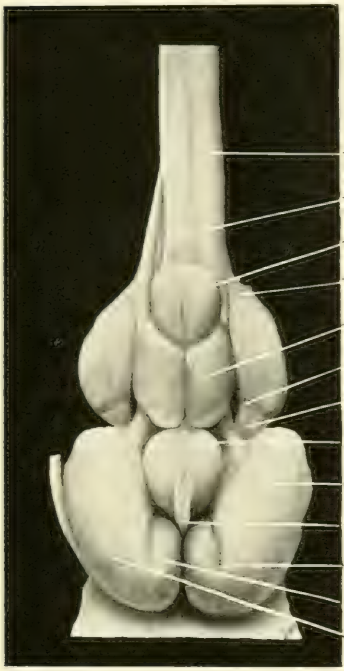
PLATE 3

EXPLANATION OF FIGURES

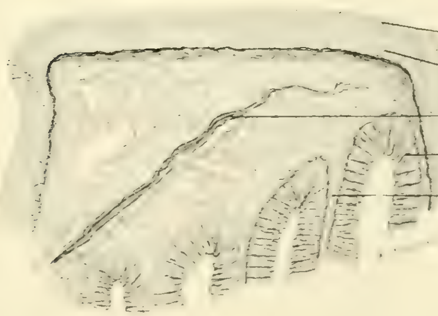
4 Ventral aspect of the rostral end of the brain. $\times$ 4.

5 Transection through the median ridge of the olfactory mucosa to show its innervation by trigeminal nerve fibers. Weigert method. $\times$ 64. In adjacent sections the medullated fibers may be seen reaching the membrana propria. Note that the epithelium of the median ridge differs from that of the remainder of the Schneiderian membrane, particularly in the large number of goblet cells present. This is also a characteristic of the respiratory epithelium of mammals as distinguished from the olfactory epithelium, which lacks almost entirely the mucus secreting cells.

chias., optic chiasma; *c. mam.*, corpus mammillare of Goldstein; *fib. trig.*, fibrae trigemini; *f. end.*, fissura endorhinalis, the sulcus rhinalis of Kappers ('06), the fovea endorhinalis externa of Kappers and Theunissen ('08), the fovea limbica of Goldstein, the fissura ectorhinalis of Owen; *go. cells.*, goblet cells; *hyp., pars gl.*, hypophysis, pars glandularis; *hyp., pars nerv.*, hypophysis, pars nervosa; *lam.*, lamella; *lob. inf.*, lobus inferior; *lob. lat.*, lobus lateralis hypothalami; *lob. med.*, lobus medius hypothalami, of which the rostral part is the tuber or tuber cinereum and the caudal the pars infundibularis; *memb. olf.*, membrana olfactoria, or olfactory portion of the Schneiderian membrane; *memb. resp.*, membrana respiratoria, the respiratory part of the Schneiderian membrane; *n. opt.*, nervus opticus; *sac. vasc.*, saccus vasculosus; *s. mam.*, sulcus mammillaris of Goldstein, separating the region of the corpus mammillare from the remainder of the lobus lateralis; *tub. post.*, tuberculum posterius.


n. opt.
chias
hyp, pars gl
tela
hyp, pars nerv
t end
tub post
lob. med
lob lat
sac vasc
s. mam
c. mam
lob inf

4


go. cells.
memb. resp.
fib. trig.
memb. olf.
lam.

5

PLATE 4

EXPLANATION OF FIGURES

6 Transection through the middle of the right olfactory bulb. Weigert method. × 31. Most of the stippled periphery is filled with the unmedullated fibers of the olfactory nerve which are ending in glomeruli in this region. An especially prominent mass of such fibers appears dorso-laterally, forming the protuberance '*a*,' as shown in fig. 1.

7 Ganglion cell of the nervus terminalis. Golgi method. × 93. See fig. 124 for the position of this cell.

8 to 12 Mitral cells of the olfactory bulb. Golgi method. × 93. In the cells from transverse sections an arrow points toward the center of the bulb; in sagittal or horizontal sections the arrow points diametrically away from the olfactory crus and toward the center of the bulb. Figs. 8 and 9 are from transverse sections, figs. 10 and 12 from longitudinal section of the bulb.

13 Fusiform cell from nucleus olfactorius anterior. Longitudinal section. Golgi method. × 93. Arrow as in figs. 8 to 12. This neurone extends diagonally across the bulb, one end entering a glomerulus.

14 Stellate cell from nucleus olfactorius anterior. Transverse section. Golgi method. × 93. Arrow as in figs. 8 to 12. Large numbers of these cells are found, most of which are connected with glomeruli; some of these glomeruli contain mitral cell dendrites, while many are small and are, apparently, formed only by stellate cell and olfactory nerve processes.

15 to 17 Neurones from the nucleus olfactorius anterior. Golgi method. × 93. Arrow placed as in figs. 8 to 12.

15 Stellate cell, connecting with a mitral cell glomerulus. From longitudinal section.

16 Stellate cell from longitudinal section. Shows one dendrite in connection with a glomerulus, while the neurite extends toward the center of the bulb.

17 Fusiform cell from longitudinal section.

dend., dendrite; *n. term.*, nervus terminalis; *neur.*, neurite; *olf. nerve*, olfactory nerve, fibers of which are scattered about the periphery at the points noted; *tr. olf. lat., pars intermed.*, tractus olfactorius lateralis, pars intermedia; *tr. olf. lat. pars. med.*, tractus olfactorius lateralis, pars medialis; *tr. olf. med., pars lat.*, tractus olfactorius medialis, pars lateralis; *tr. olf. med., pars. med.*, tractus olfactorius medialis, pars medialis.

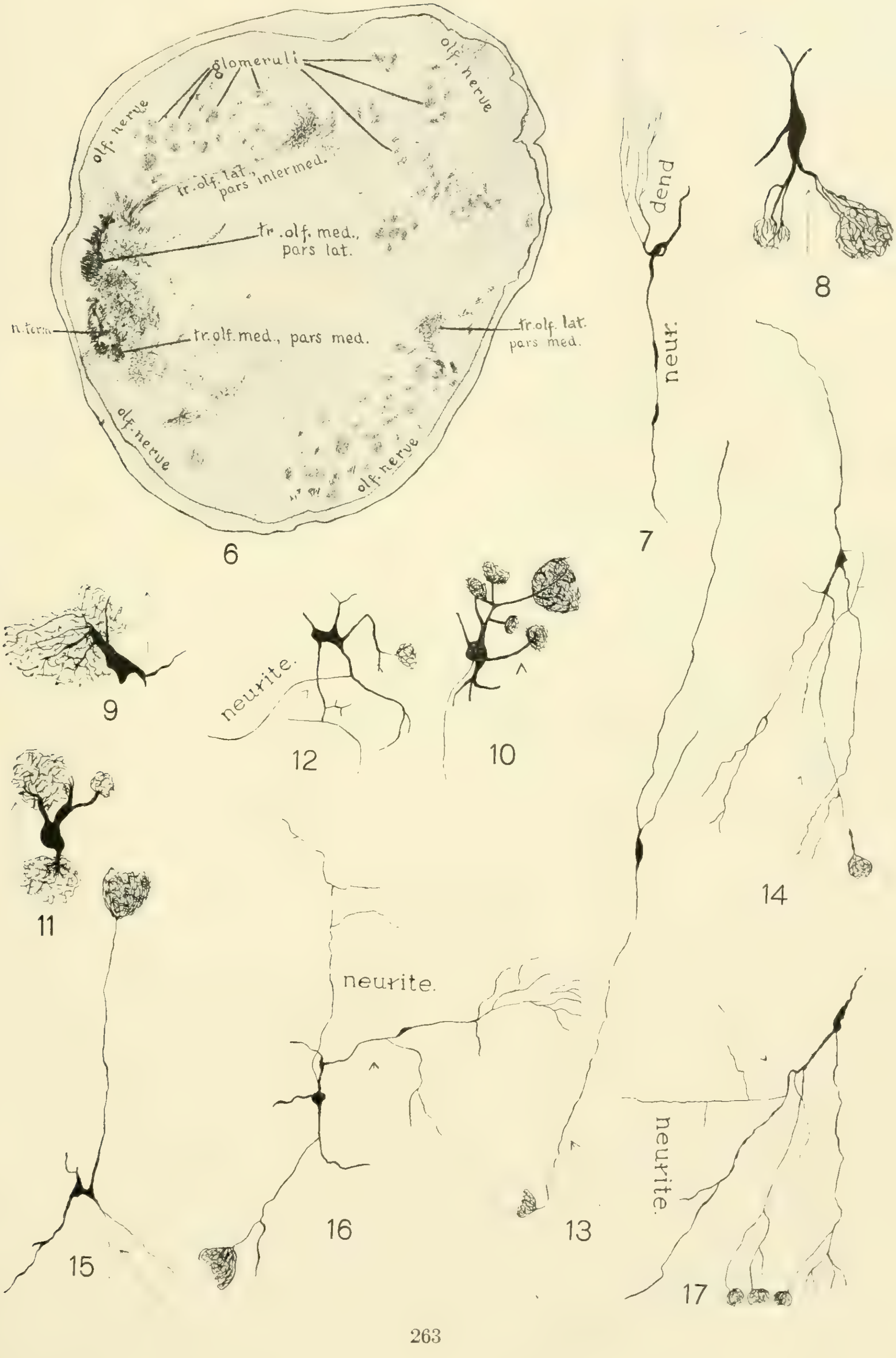

glomeruli
olf. nerve
olf. nerve
tr. olf. lat., pars intermed.
tr. olf. med., pars lat.
n. term.
tr. olf. med., pars med.
tr. olf. lat. pars med.
olf. nerve
olf. nerve
6
dend
neur.
7
8
9
neurite.
12
10
11
14
neurite.
16
13
15
neurite.
17

PLATE 5

EXPLANATION OF FIGURES

18 to 20 Neurones from the nucleus olfactorius anterior. Golgi method. × 93. Arrow placed as in figs. 8 to 12.

18 Goblet shaped cell from longitudinal section.

19 Fusiform granule cell from longitudinal section.

20 Stellate granule cell from longitudinal section.

21 Fusiform cell from sagittal section of the olfactory bulb, showing neurite entering the crus. Golgi method. × 93.

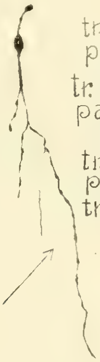
22 Transection through the middle of the right olfactory crus. Weigert method. × 33. This section was drawn to show, particularly, the nervus terminalis; the remaining fiber pathways do not come out so clearly as in other series.

23 Transection through the caudal part of the right olfactory crus, immediately rostral to the cerebral hemispheres. Weigert method. × 33.

24 Transection through the rostral portion of the cerebral hemispheres. Weigert method. × 17. This section shows the relation to the hemispheres, of the tracts of the crura.

bulb. olf., bulbus olfactorius; *crus olf.*, crus olfactorium; *f. endorh.*, fissura endorhinalis; *n. term.*, nervus terminalis; *neur.*, neurite; *tr. olf. asc.*, tractus olfactorius ascendens, the radix olfactoria medialis propria of Kappers; *tr. olf. asc., pars lat.*, tractus olfactorius ascendens, pars lateralis; *tr. olf. asc., pars. med.*, tractus olfactorius ascendens, pars medialis; *tr. olf. lat., pars intermed.*, tractus olfactorius lateralis, pars intermedia; *tr. olf. lat., pars lat.*, tractus olfactorius lateralis, pars lateralis; *tr. olf. lat., pars. med.*, tractus olfactorius lateralis, pars medialis; *tr. olf. med., pars lat.*, tractus olfactorius medialis, pars lateralis; *tr. olf. med., pars med.*, tractus olfactorius medialis, pars medialis.

18



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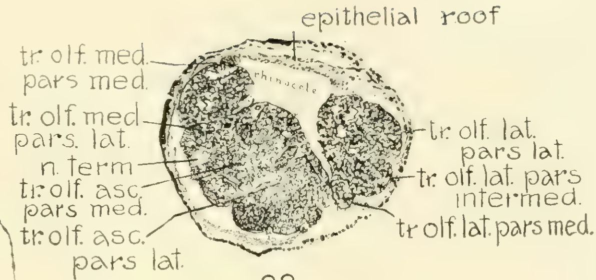

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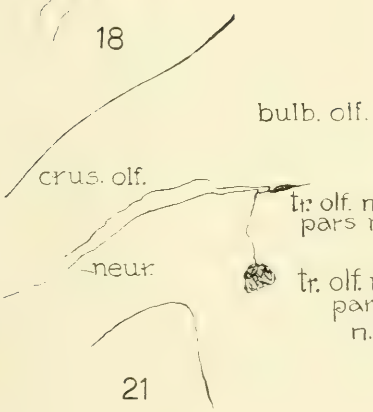

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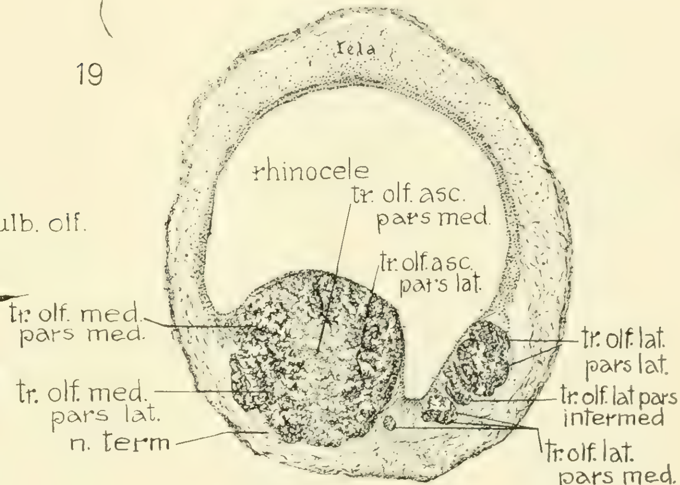

23

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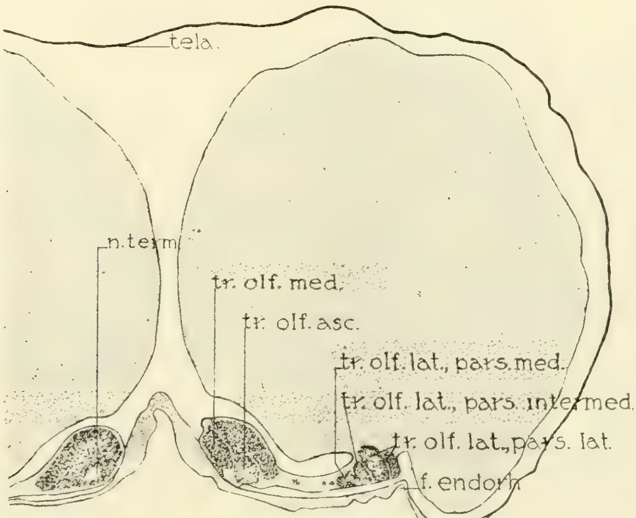


24

PLATE 6

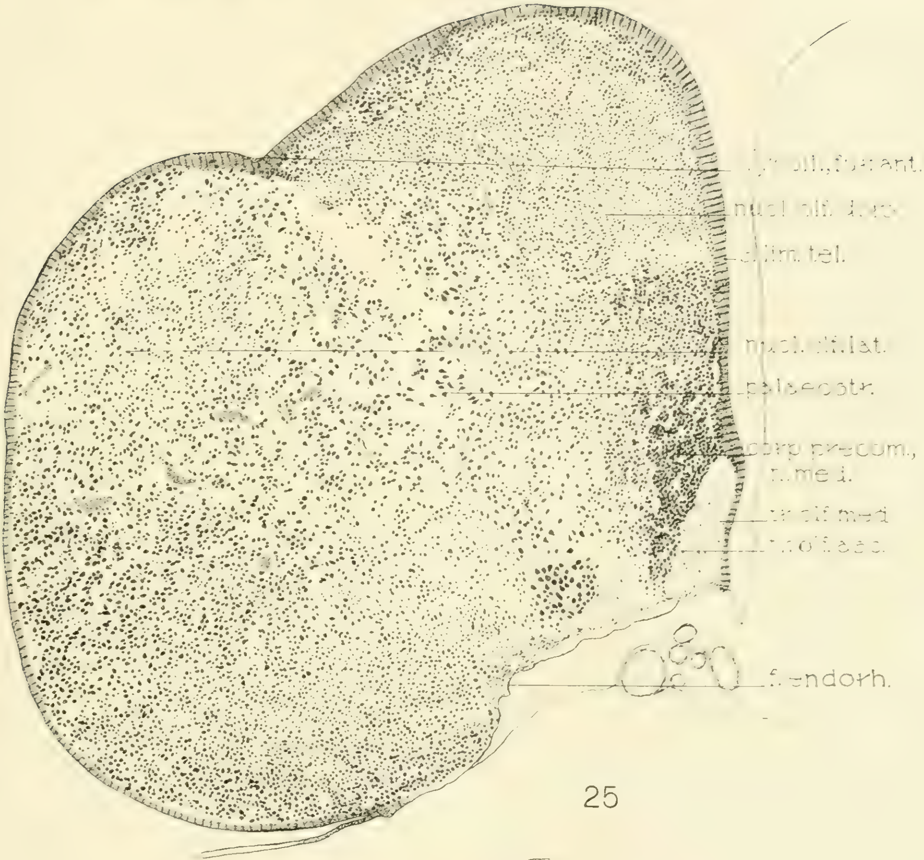
EXPLANATION OF FIGURES

25 Transection through the rostral portion of the left cerebral hemisphere, slightly caudal to fig. 24. Toluidin blue method. × 46.

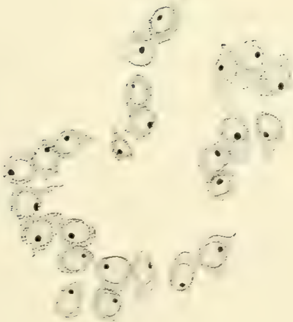
26 Cells of the nucleus medianus. Toluidin blue method. × 575. From transection. This nucleus is characterized by the arrangement of the cells in small, closely packed groups as shown in the figure. Compare fig. 25.

27 Oblique longitudinal section through the hemispheres showing the origin of the centrifugal fibers of the tractus olfactorius ascendens. Golgi method. × 9. The section is much nearer the frontal than the sagittal plane, as is shown by the inclusion of both olfactory crura, a portion of both optic nerves and much of the anterior commissure.

com. ant., commissura anterior; *corp. precom.*, *n. med.*, corpus precommissurale, nucleus medianus, this latter is the rostral end of the group of cells called 'lobus olfactorius posterior, pars medialis' by Goldstein; 'area olfactoria posterior medialis' and 'epistriatum' by Kappers ('06); 'area praecommissuralis septi' by Kappers and Theunissen ('08); 'ganglion mediale septi' by Gaupp ('99) and 'paraterminal body' by Elliot Smith ('03); *crura olf.*, crura olfactoria; *f. endorh.*, fissura endorhinalis; *hyp.*, hypophysis; *n. opt.*, nervus opticus; *nucl. olf. dors.*, nucleus olfactorius dorsalis; *nucl. olf. lat.*, nucleus olfactorius lateralis; *palaeostr.*, palaeostriatum; *s. lim. tel.*, sulcus limitans telencephali; *s. ypsil.*, *fur. ant.*, sulcus ypsiliformis, furca anterior, the fovea endorhinalis interna of Kappers; *tr. olf. asc.*, tractus olfactorius ascendens; *tr. olf. med.*, tractus olfactorius medialis.


nucl. olf. lat.
palaeostr.
corp. precom.,
f. endorh.

25



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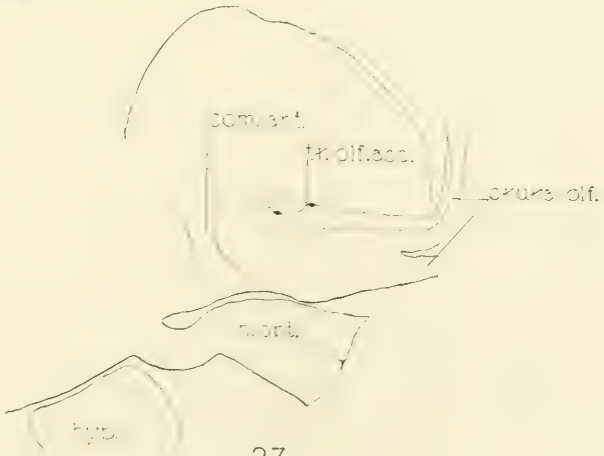

com. ant.
tr. olf. asc.

27

PLATE 7

EXPLANATION OF FIGURES

28–30 Cells of origin of the fibers of the tractus olfactorius ascendens. Golgi method. × 93. These cells lie in the rostral portion of the precommissural body, in the nucleus medianus (see fig. 27). They are taken from a sagittal series and show the branching of the dendrites among the cells of the nucleus. The neurites terminate in the nucleus olfactorius anterior of the olfactory bulb.

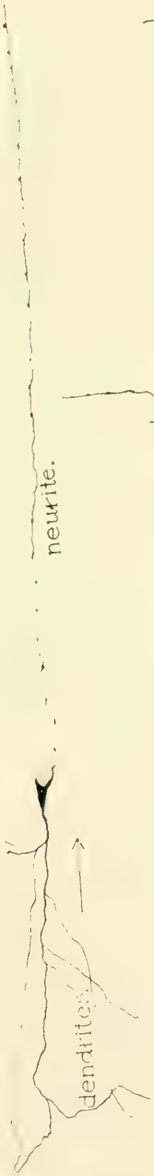
31 Association cell of the nucleus medianus. Golgi method. × 93. From sagittal section.

32–33 Cells of the rostral part of the nucleus olfactorius lateralis. Golgi method. × 93. The neurite of fig. 32 enters the tractus strio-thalamicus, while that of fig. 33 apparently ends in the ventro-lateral portion of the hemisphere. The cells are taken from a sagittal series and occupy a position about midway between the dorsal and ventral surfaces of the hemisphere.

34 Transection through the cerebral hemispheres immediately rostral to the anterior commissure. Weigert method. × 17. The nervus terminalis is, at this level, separated from the tractus olfactorius medialis, preparatory to its decussation; the two components of the tractus olfactorius medialis likewise appear distinctly.

com. interbulb., commissura interbulbaris; *f. end.*, fissura endorhinalis; *n. term.*, nervus terminalis; *s. ypsil.*, *ant. limb*, sulcus ypsiliformis, anterior limb; *tr. olf. lat.*, tractus olfactorius lateralis spreading in the form of a crescent to end in the nucleus olfactorius lateralis, *tr. olf. med.*, *pars lat.*, tractus olfactorius medialis, pars lateralis; *tr. olf. med.*, *pars med.*, tractus olfactorius medialis, pars medialis; *tr. strio-thal.*, tractus strio-thalamicus; bundles appearing here are made up, mainly, of fibers which do not decussate.

28



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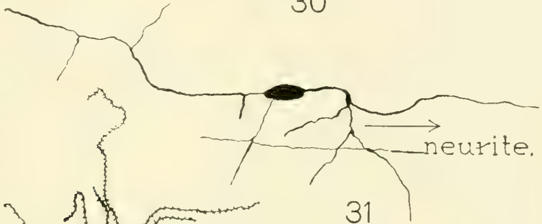
30



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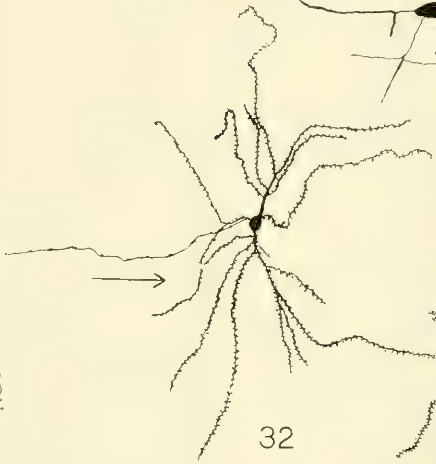
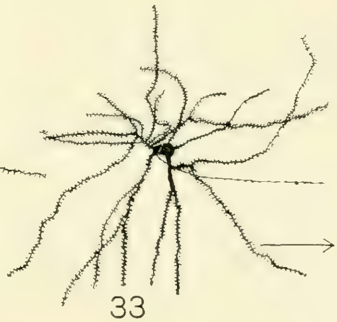


32



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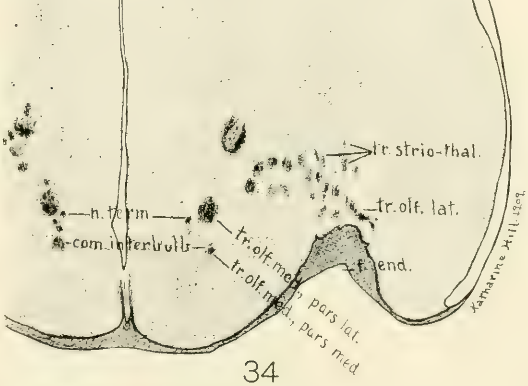


34

PLATE 8

EXPLANATION OF FIGURES

35 Transection through the hemispheres at the rostral margin of the anterior commissure. Weigert method. × 17. This section shows particularly the decussation of the nervus terminalis (see also Sheldon '09, figs. 6 and 7); also the commissura dorsalis, partly made up of fibers connecting the two partes supracommissurales of the precommissural body, partly of fibers connecting the two nuclei olfactorii dorsales (commissura hippocampi, pars anterior). The relation of the corpus precommissurale to the anterior commissure is brought out clearly, the pars commissuralis or commissure bed of Elliot Smith appearing ventrally and the pars supracommissuralis dorsally.

36 Transection through the middle of the anterior commissure. Weigert method. × 17. Shows especially the decussation of the tractus strio-thalamicus cruciatus and of the tractus olfactorius medialis, pars lateralis; also the commissura hippocampi, pars posterior, presenting points of similarity, morphologically, with the commissura dorsalis of Elliot Smith in amphibians, reptiles and mammals, the commissura pallii of Kappers and Theunissen in amphibians, the commissura pallii posterior of Edinger in reptiles, the commissura olfactorii internuclearis of Goldstein, and connecting the nuclei pyriformes of the two sides. This section also brings out several of the components of the medial forebrain bundle, the tractus olfacto-lobaris medialis of Kappers ('06). Dorsally, mingled with the fibers of the commissura dorsalis are the fibers of the tractus hypothalamo-olfactorius medialis. These are ascending fibers, part of which decussate in the anterior commissure and part in the region of the nucleus posterior tuberis (see figs. 102, 104, 105). This is the tractus olfacto-hypothalamicus medialis of Goldstein.

com. ant., commissura anterior; *com. dors.*, commissura dorsalis; *com. dors.+ dec. tr. hyp. olf. med.*, commissura dorsalis plus decussatio tractorum hypothalamo-olfactoriorum medialium; *com. hipp., pars post.*, commissura hippocampi, pars posterior; *com. interbulb.* (*tr. olf. med., pars. med.*), commissura interbulbaris (tractus olfactorius medialis, pars medialis, the fibers of the tract forming the commissura interbulbaris (aut); *corp. precom., pars com.*, corpus precommissurale, pars supracommissuralis; *dec. tr. olf. med., pars lat.*, decussation of the tractus olfactorii mediales, partes laterales; *dec. tr. strio-thal. cruc.*, decussation of the tractus strio-thalamici cruciati; *n. olf. lat.*, nucleus olfactorius lateralis; *n. opt.*, nervus opticus; *n. pyr.*, nucleus pyriformis—this, together with a part of the nucleus olfactorius lateralis, corresponds to the lobus olfactorius posterior or area olfactoria posterior lateralis of Kappers ('06), the lobus olfactorius posterior, pars lateralis of Goldstein, the lobus olfactorius of Edinger ('08), the area olfactoria lateralis of Kappers and Theunissen; *n. term.*, nervus terminalis; *palaeostr.*, palaeostriatum; *s. lim. tel.*, sulcus limitans telencephali; *s. ypsil., fur. ant.*, sulcus ypsiliformis, furca anterior; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. lat.*, tractus olfactorius lateralis; *tr. olf. med., pars. lat.*, tractus olfactorius medialis, pars lateralis; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis—this latter component of the medial forebrain bundle is descending and made up of uncrossed fibers (see fig. 136), which, with the pars intermedia, and pars dorsalis, form the tractus olfacto-lobaris medialis or tractus olfacto-hypothalamicus medialis of Kappers; *tr. strio-thal.*, tractus strio-thalamicus.


s. lim. tel
s. ypsil. fur. ant.
palaeostr.
tr. strio-thal.
corp. precom.,
pars. supracom.
tr. olf. lat.
com. dors.
tr. olf. med., pars.
lat.
n. term.
corp. precom., pars.
com.
com. interbulb, [tr. olf.
med., pars. med.]
com. ant.

35


n. olf. lat.
palaeostr.
corp. precom.,
pars supracom.
n. pyr.
tr. hyp. olf. med.
com. dors. + dec.
tr. hyp. olf. med.
com. hipp., pars post
tr. olf. thal. med.,
pars vent.
dec. tr. olf. med.,
pars lat.
dec. tr. strio-thal.
cruc.
corp. precom.,
pars com.
com. ant.
n. opt

36

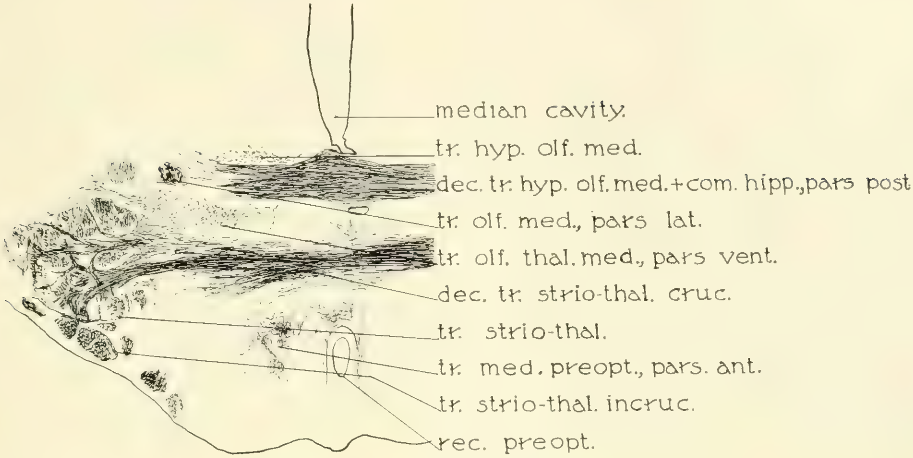
PLATE 9

EXPLANATION OF FIGURES

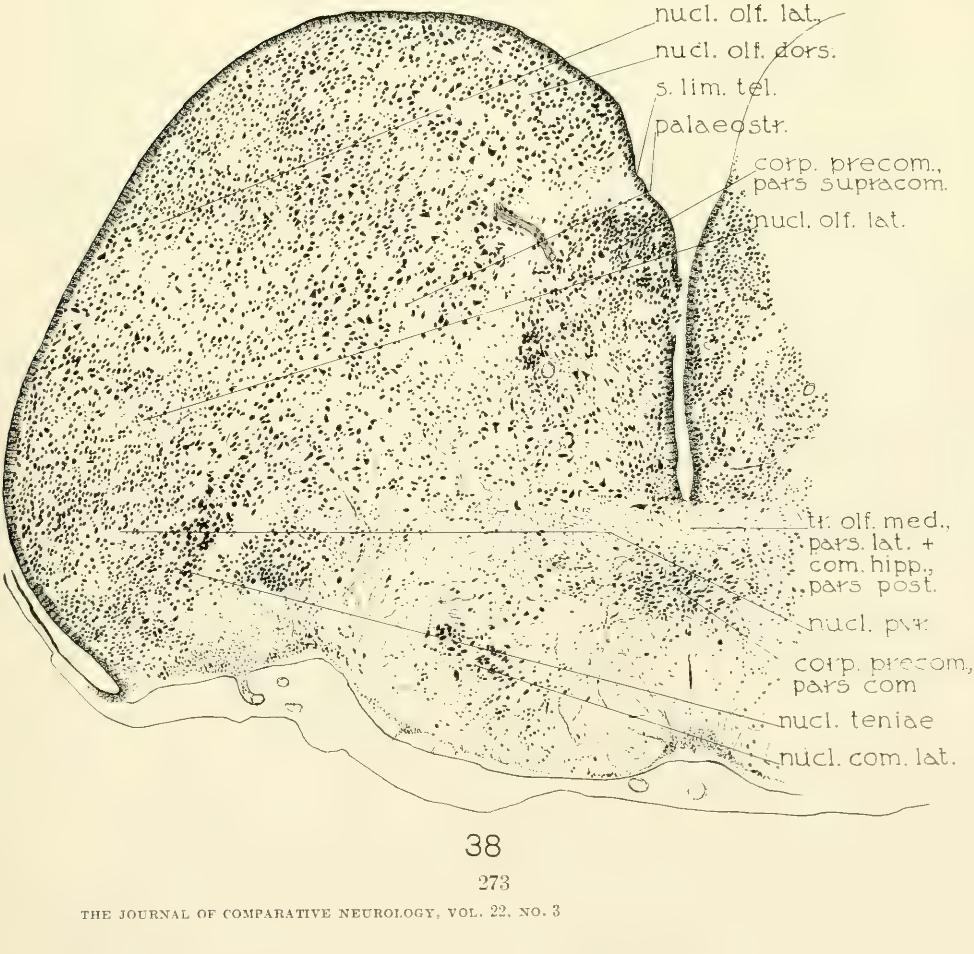
37 Transection through the anterior commissure. Ramón y Cajal method. $\times$ 46. Shows particularly the decussation of the tractus strio-thalamicus cruciatus.

38 Transection through the anterior commissure. Toluidin blue method. $\times$ 46. Shows with great clearness the limits of the pars supracommissuralis of the corpus precommissurale, the sulcus limitans telencephali, the nucleus olfactorius dorsalis and the bed of the anterior commissure, the pars commissuralis of the precommissural body.

corp. precom., pars com., corpus precommissurale, pars commissuralis; *corp. precom., pars supracom.*, corpus precommissurale, pars supracommissuralis; *dec. tr. hyp. olf. med. + com. hipp., pars post.*, decussatio tractus hypothalamo-olfactorii medialis plus commissura hippocampi, pars posterior; *dec. tr. strio-thal. cruc.*, decussatio tractus strio-thalamici cruciati; *necl. com. lat.*, nucleus commissuralis lateralis; *nucl. olf. dors.*, nucleus olfactorius dorsalis; *nucl. olf. lat.*, nucleus olfactorius lateralis; *nucl. pyr.*, nucleus pyriformis; *nucl. teniae*, nucleus teniae, the nucleus taeniae of Edinger, Kappers and Goldstein; *palaeostr.*, palaeostriatum; *rec. preopt.*, recessus preopticus; *s. lim. tel.*, sulcus limitans telencephali; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. med., pars. lat.*, tractus olfactorius medialis, pars lateralis; *tr. olf. med., pars lat. + com. hipp., pars post.*, tractus olfactorius medialis, pars lateralis plus commissura hippocampi, pars posterior; *tr. olf. that. med., pars. vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. med. preopt., pars. ant.*, tractus medianopreopticus, pars anterior; *tr. strio-thal.*, tractus strio-thalamicus; *tr. strio-thal. incruc.*, tractus strio-thalamicus incruciatus.


median cavity.
tr. hyp. olf. med.
dec. tr. hyp. olf. med.+com. hipp., pars post
tr. olf. med., pars lat.
tr. olf. thal. med., pars vent.
dec. tr. strio-thal. cruc.
tr. strio-thal.
tr. med. preopt., pars. ant.
tr. strio-thal. incruc.
rec. preopt.

37


nucl. olf. lat.
nucl. olf. dors.
s. lim. tel.
palaeostr.
corp. precom., pars supracom.
nucl. olf. lat.
tr. olf. med., pars. lat. + com. hipp., pars post.
nucl. pvr.
corp. precom., pars com
nucl. teniae
nucl. com. lat.

38

PLATE 10

EXPLANATION OF FIGURES

39 Sagittal section through the hemisphere. Golgi method. × 9. Shows cells of the corpus precommissurale sending axones, the fibrae precommissurales striaticae, into the palaeostriatum. Both ascending and descending fibers of the tractus strio-thalamicus are shown; note especially the dichotomous branching of the ascending neurites to form tangential fibers.

40 Cell of origin of the tractus olfacto-thalamicus medialis from the nucleus medianus. Golgi method. × 94. From sagittal section.

41–42 Association cells of the nucleus medianus. Golgi method. × 93. From sagittal section.

43 One of the neurones of the nucleus medianus, the neurite of which forms one of the fibrae precommissurales striaticae. Golgi method. × 93. (See fig. 39.) From sagittal section.

44 Cells of the palaeostriatum. Toluidin blue method. × 575. From transverse section.

45 Neurone from cerebral hemisphere. Golgi method. × 93. From sagittal section, neurite directed caudad, into tractus strio-thalamicus. This neurone can hardly be assigned to a definite region, as it lies about midway between the typical cells of the palaeostriatum and those of the area olfactoria lateralis. It will be noted that its perikaryon is larger than that of typical cells of the area olfactoria lateralis but that its dendrites do not show the conspicuous thorns of the typical palaeostriatal neurones.

46 Cells of the dorsal portion of the corpus precommissurale, pars supracommissuralis. Toluidin blue method. From transection. × 575.

47 Cells of the nucleus pyriformis. Toluidin blue method. From transection. × 575.

48 Neurone from the nucleus olfactorius lateralis. Golgi method. × 93. From sagittal section. This cell is found in the dorsal portion of the hemisphere, adjacent to the nucleus olfactorius dorsalis, but in the region called epistriatum by Johnston; its neurite enters the tractus strio-thalamicus.

49 Neurone from the nucleus olfactorius lateralis. Golgi method. × 93. From sagittal section. This is found in the rostro-lateral portion of the hemisphere; it comes into association with the ascending fibers of the tractus thalamo-striaticus as shown also in figs. 50 and 51.

fib. precom. str., fibrae precommissurales striatici; *hab.*, habenula; *tr. strio-thal.*, tractus strio-thalamicus; *tr. thal. striat.*, tractus thalamo-striaticus.

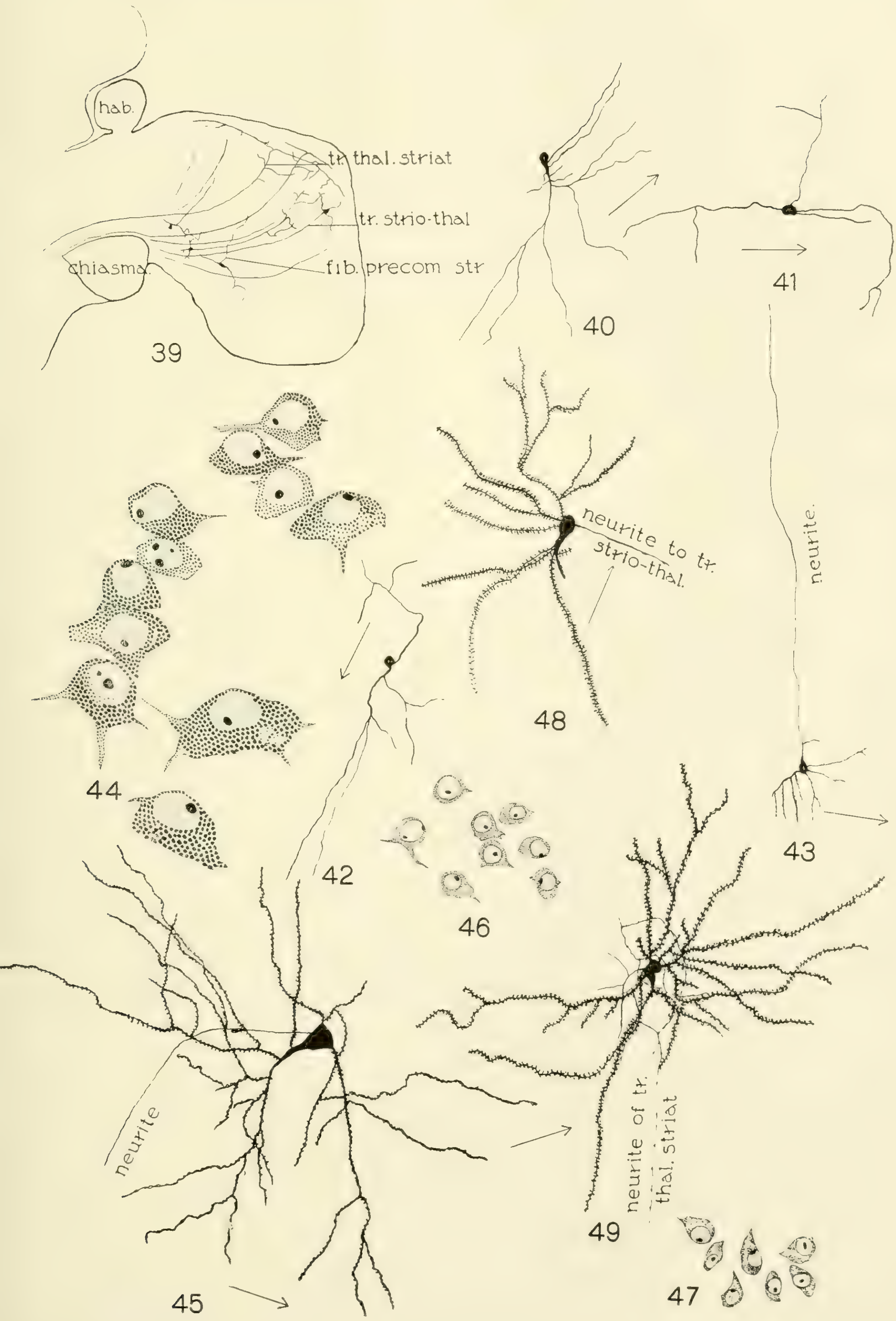

hab.
tr. thal. striat
tr. strio-thal
chiasma
fib. precom str
39
40
41
neurite to tr. strio-thal.
neurite.
48
44
42
46
43
neurite
neurite of tr. thal. striat
49
45
47

PLATE 11

EXPLANATION OF FIGURES

50–51 Neurones of the palaeostriatum. Golgi method. × 185. From sagittal sections. About the perikaryon of each neurone is a network formed by the terminal arborization of the neurites of the ascending fibers of the tractus strio-thalamicus. The larger portion of the cells of the palaeostriatum are very thorny, as shown in the cells drawn; a number of the neurones, however, particularly those giving rise to descending fibers of the tractus strio-thalamicus, resemble more closely fig. 45, with less conspicuous thorns and a larger perikaryon. The cells shown in figs. 50 and 51 are evidently association cells; their processes extend over a very large area, bringing different parts of the hemispheres into relation with one another, with the thalamus and hypothalamus, and with the olfactory apparatus through the precommissural body.

tr. thal. striat., tractus thalamo-striaticus.

50

51

PLATE 12

EXPLANATION OF FIGURES

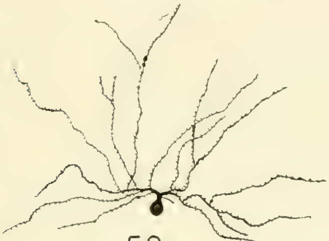
52 Neurone from the nucleus olfactorius lateralis. Golgi method. × 93. From sagittal section. This cell is found in the dorso-lateral portion of the hemisphere, its neurite enters the tractus strio-thalamicus.

53 Neurone of the nucleus pyriformis. Golgi method. × 93. From sagittal section. Most of the neurites from cells of this character and location enter the tractus olfacto-hypothalamicus lateralis.

54 Transection through the region of the recessus preopticus. Ramón y Cajal method. × 46.

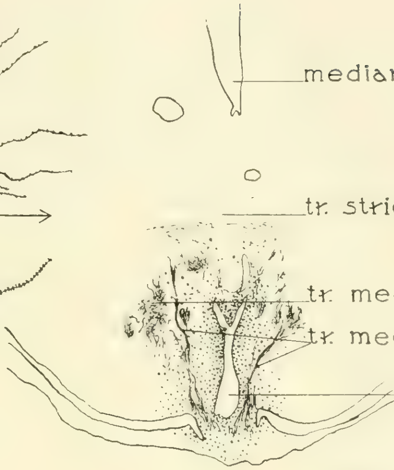
55 Transection through the caudal part of the anterior commissure. Weigert method. × 17.

corp. precom., pars supracom., corpus precommissurale, pars supracommissuralis; *n. opt.*, nervus opticus; *nucl. com. lat.*, nucleus commissuralis lateralis; *rec. preopt.*, recessus preopticus; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. med. preopt., pars ant.*, tractus mediano-preopticus, pars anterior, consisting of fibers originating in the anterior part of nucleus medianus and terminating about the recessus preopticus; *tr. med. preopt., pars post.*, tractus mediano-preopticus, pars posterior, consisting largely of fibers originating in the commissure bed and ending about the third ventricle in the region of the nucleus preopticus; *tr. olf. med., pars lat. +c. hipp., pars post.*, tractus olfactorius medialis, pars lateralis plus commissura hippocampi, pars posterior; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; this component of the medial forebrain bundle appears in this section for the first time in the drawings; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. strio-thal. cruc.*, tractus strio-thalamicus cruciatus; *tr. strio-thal. incruc.*, tractus strio-thalamicus incruciatus; *tr. ten.*, tractus teniae, the tractus olfacto-habenularis of Kappers, Goldstein, etc.



52

53


median cavity.
tr. strio-thal. cruc.
tr. med. preopt., pars ant.
tr. med. preopt., pars post.
rec. preopt.

54


corp. precom., pars supracom.
tr. ten.
tr. olf. thal. med., pars dors.
tr. hyp. olf. med.
tr. olf. med., pars lat.+ c. hipp., pars post.
tr. olf. thal. med., pars vent.
tr. strio-thal. cruc.
tr. strio-thal. incruc.
nucl. com. lat.
n. opt.

55

PLATE 13

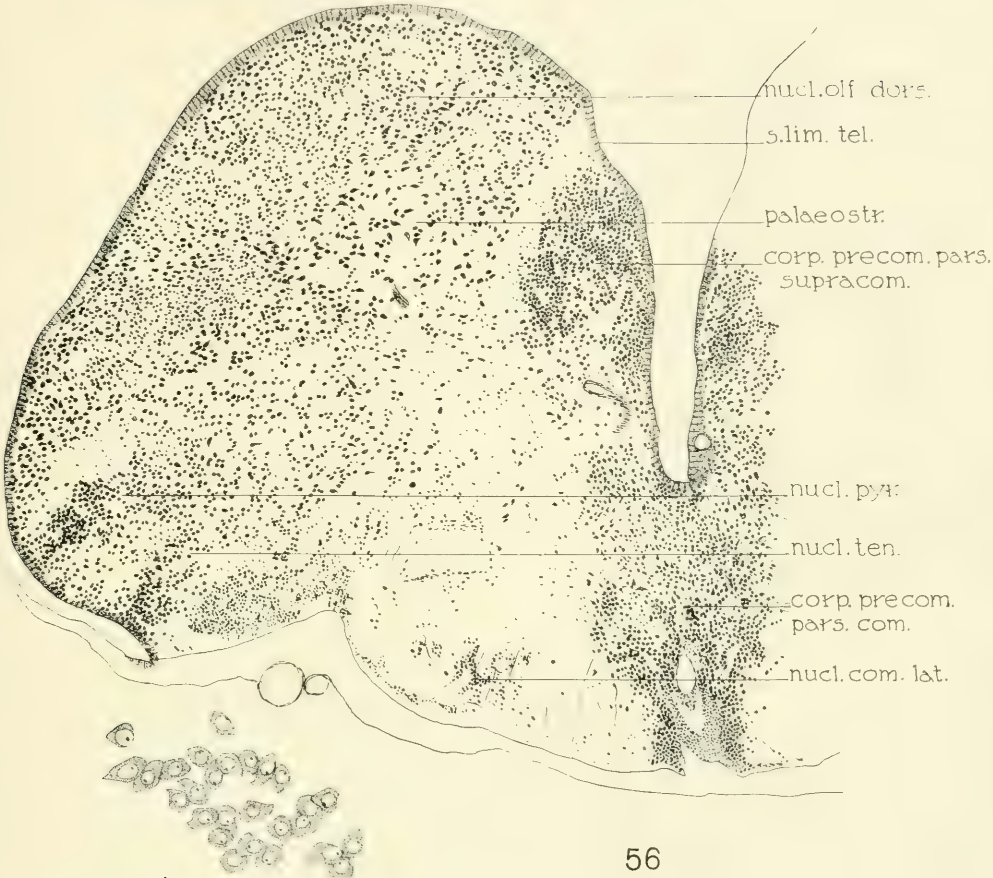
EXPLANATION OF FIGURES

56 Transection through the caudal part of the anterior commissure. Toluidin blue method. × 46.

57 Cells of the nucleus teniae. Toluidin blue method. From transverse section. × 575.

58–60 Neurones of the nucleus teniae. Golgi method. × 93. From oblique sections, about midway between sagittal and transverse.

corp. precom., pars com., corpus precommissurale, pars commissuralis; *corp. precom., pars supracom.*, corpus precommissurale, pars supracommissuralis; *nucl. com. lat.*, nucleus commissuralis lateralis; *nucl. olf. dors.*, nucleus olfactorius dorsalis; *nucl. pyr.*, nucleus pyriformis; *nucl. ten.*, nucleus teniae; *palaeostr.*, palaeostriatum; *s. lim. tel.*, sulcus limitans telencephali.


nucl. olf dors.
s. lim. tel.
palaeostr.
corp. precom. pars. supracom.
nucl. pyr.
nucl. ten.
corp. precom. pars. com.
nucl. com. lat.

56

57

58

59

60

PLATE 14

EXPLANATION OF FIGURES

61 Transection through the caudal part of the anterior commissure. Weigert method. × 17.

62 Sagittal section slightly to one side of the median line, showing the tractus preopticus superior. Golgi method. × 46. The location of the different parts of the nucleus preopticus is indicated by broken lines.

63 Neurone of the tractus preopticus superior. Golgi method. × 93. From sagittal section. (See fig. 62).

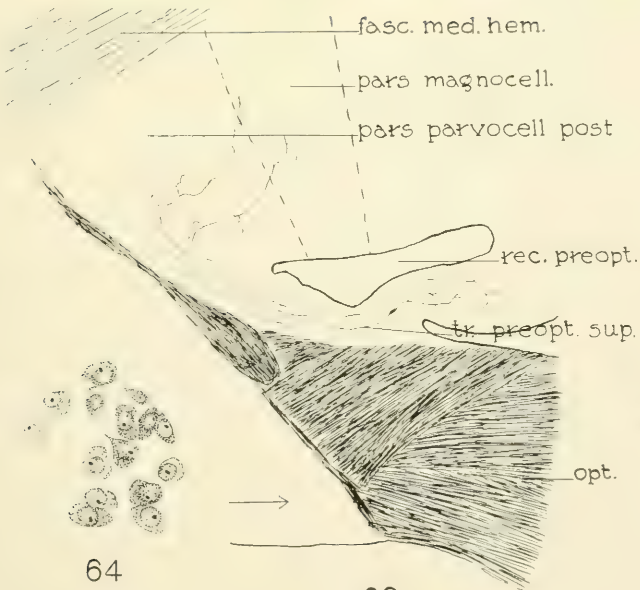
64 Cells of the nucleus preopticus, pars parvocellularis anterior. Toluidin blue method. × 575.

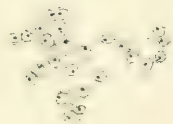
65 Cells of the nucleus entopeduncularis. Toluidin blue method. × 575.

corp. precom., pars com., corpus precommissurale, pars commissuralis; *corp. precom., pars supracom.*, corpus precommissurale pars supracommissuralis; *fasc. med. hem.*, fasciculus medialis hemisphaerii; *n. opt.*, nervus opticus; *nucl. preopt., pars parvocell.*, nucleus preopticus, pars parvocellularis; *opt.*, optic chiasma; *pars magnocell.*, pars magnocellularis of the nucleus preopticus; *pars parvocell. post.*, pars parvocellularis posterior of the nucleus preopticus; *rec. preopt.*, recessus preopticus. *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. med. preopt., pars ant.*, tractus mediano-preopticus, pars anterior; *tr. preopt. sup.*, tractus preopticus superior; the connections of this tract are fully shown in the figure; it is apparently, Goldstein's tract × (Taf. 11, fig. 7); *tr. strio-thal. incruc.*, tractus strio-thalamicus incruciatus; *tr. ten.*, tractus teniae.


corp. precom., pars supracom
tr. ten.
tr. olf. thal. med., pars. vent.
tr. olf. thal. med., pars dors.
tr. hyp. olf. med.
corp. precom., pars. com
tr. strio-thal. incruc.
tr. med. preopt., pars. ant
nucl preopt pars. parvocell.
n. opt.

61


fasc. med. hem.
pars magnocell.
pars parvocell post
rec. preopt.
tr. preopt. sup.
opt.



65

64

62

63

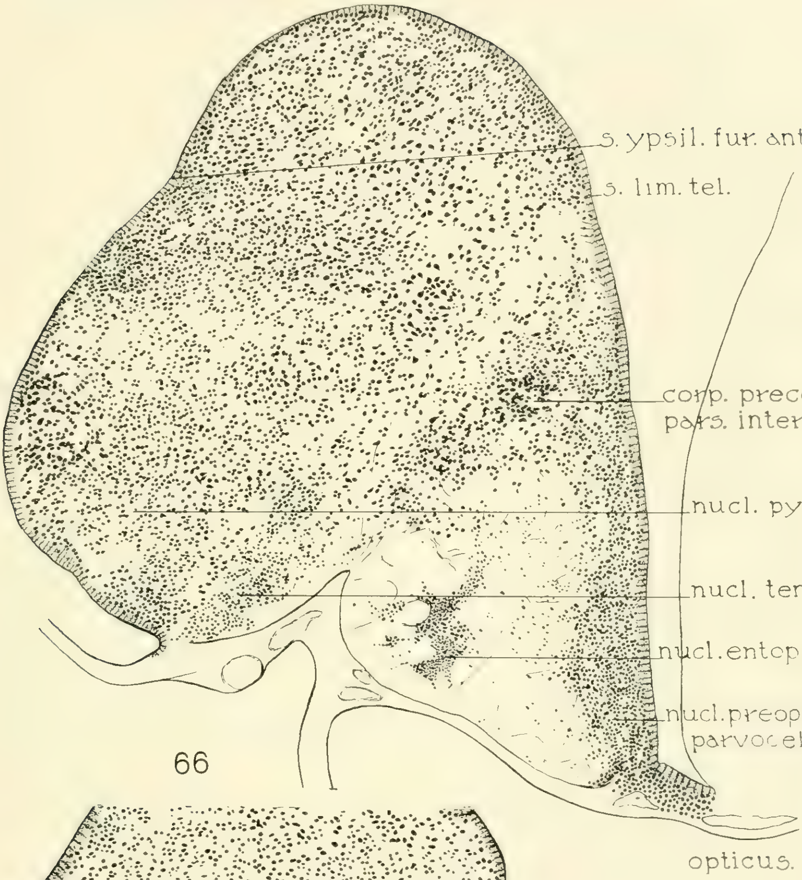
PLATE 15

EXPLANATION OF FIGURES

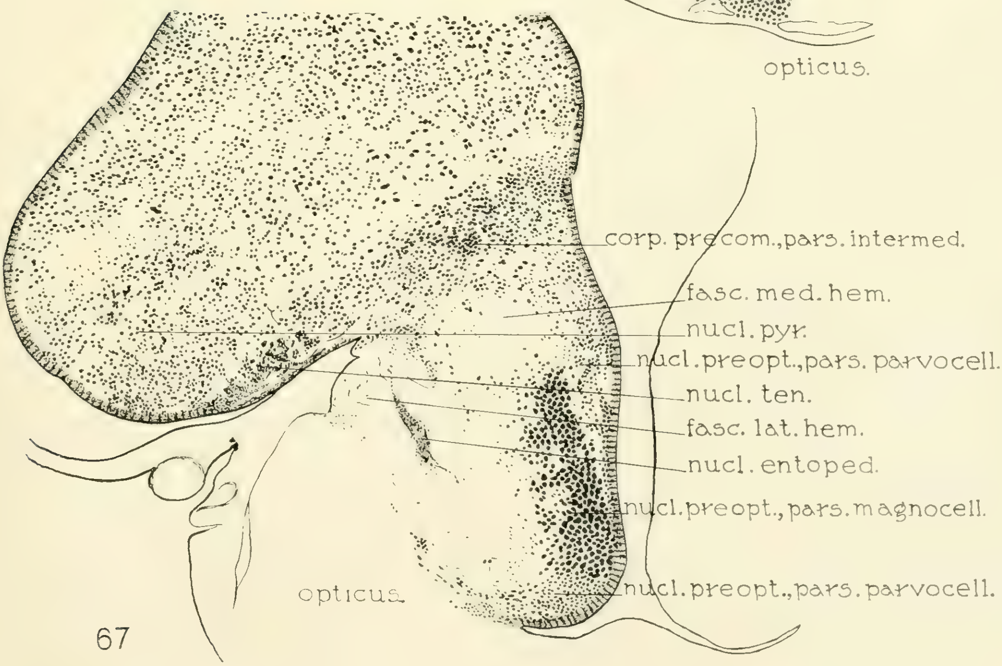
66 Transection through the cerebral hemispheres caudal to the anterior commissure. Toluidin blue method. × 46.

67 Transection through the cerebral hemispheres slightly caudal to the level shown in fig. 66. Toluidin blue method. × 46. Shows particularly the rostral portion of the pars magnocellularis of the nucleus preopticus and its relation to the pars parvocellularis.

corp. precom., pars intermed., corpus precommissurale, pars intermedia; this, the caudal prolongation of the pars supracommissuralis, meets the nucleus pyriformis at the posterior pole of the hemisphere, it likewise comes in close contact with the extension of the pars commissuralis caudally and ventrally, the pars parvocellularis of the nucleus preopticus; *fasc. lat. hem.*, fasciculus lateralis hemisphaerii, the lateral forebrain bundle; consisting of the tractus strio-thalamicus, tractus thalamo-striaticus, tractus olfacto-hypothalamicus lateralis and tractus hypothalamo-olfactorius lateralis; *fasc. med. hem.*, fasciculus medialis hemisphaerii, the medial forebrain bundle; *nucl. entoped.*, nucleus entopeduncularis; *nucl., preopt. pars magnocell.*, nucleus preopticus, pars magnocellularis; *nucl. preopt., pars parvocell.*, nucleus preopticus, pars parvocellularis anterior; *nucl. pyr.*, nucleus pyriformis; *nucl. ten.*, nucleus teniae; *s. lim. tel.*, sulcus limitans telencephli; *s. ypsil., fur. ant.*, sulcus ypsiliformis, furca anterior.


s. ypsil. fur. ant.
s. lim. tel.
corp. precom. pars. intermed.
nucl. pyr.
nucl. ten.
nucl. entoped.
nucl. preopt., pars. parvocell. ant.
opticus.

66


corp. precom., pars. intermed.
fasc. med. hem.
nucl. pyr.
nucl. preopt., pars. parvocell.
nucl. ten.
fasc. lat. hem.
nucl. entoped.
nucl. preopt., pars. magnocell.
nucl. preopt., pars. parvocell.
opticus.

67

PLATE 16

EXPLANATION OF FIGURES

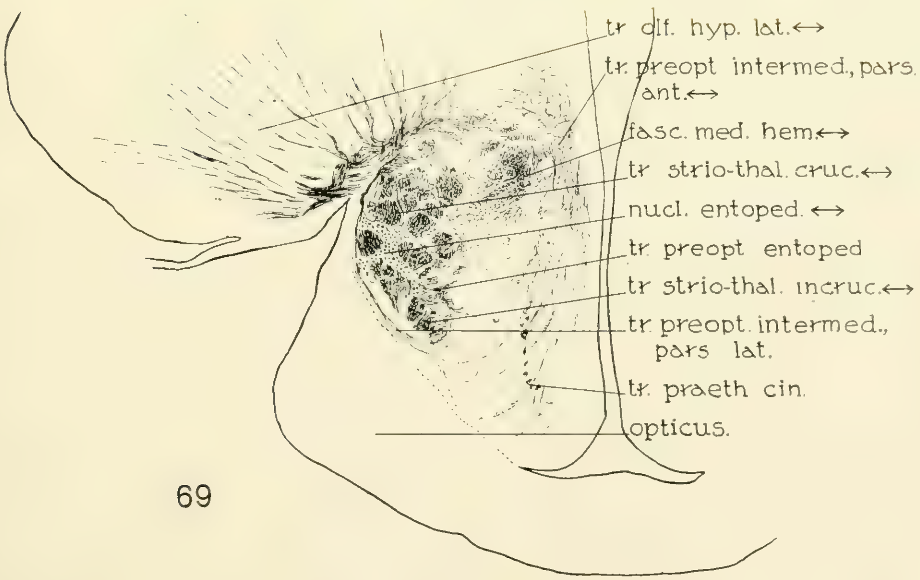
68 Transection, at the level of the chiasma. Weigert method. × 17.

69 Transection at approximately the same level as shown in fig. 68. Ramón y Cajal method. × 46.

corp. precom., pars intermed., corpus precommissurale, pars intermedia; *fasc. lat. hem.* ⟵⟶, fasciculus lateralis hemisphaerii; *fasc. med. hem.* ⟵⟶, fasciculus medialis hemisphaerii; *nucl. entoped.*, nucleus entopeduncularis; *nucl. preopt., pars magnocell,*, nucleus preopticus, pars magnocellularis; *nucl. preopt., pars parvocell.*, nucleus preopticus, pars parvocellularis; *nucl. pyr.*, nucleus pyriformis; *sac. dors.*, saccus dorsalis; *s. ypsil., fur. post.*, sulcus ypsiliformis, furca posterior; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. hyp. lat.* ⟵⟶, tractus olfacto-hypothalamicus lateralis; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. opt.*, tractus opticus; *tr. praeth. cin.*, tractus praethalamo-cinereus of Kappers; *tr. preopt. entoped.* ⟵⟶, tractus preoptico-entopeduncularis; *tr. preopt. intermed., pars ant.* ⟵⟶, tractus preoptico-intermedius, pars anterior; *tr. preopt. intermed., pars lat.*, tractus preoptico-intermedius, pars lateralis; *tr. strio-thal. cruc.* ⟵⟶, tractus strio-thalamicus cruciatus; *tr. strio-thal. incruc.* ⟵⟶, tractus strio-thalamicus incruciatus; *tr. ten.*, tractus teniae.


sac. dors.
s. ypsil., fur. post.
corp. precom., pars intermed.
nucl. pyr.
tr. ten.
tr. olf. thal. med., pars dors.
tr. hyp. olf. med.
tr. olf. thal. med., pars vent.
fasc. lat. hem. ↔
nucl. entoped.
nucl. preopt., pars magnocell.
tr. preopt. intermed., pars lat.
nucl. preopt., pars parvocell.
tr. opt.

68


tr olf. hyp. lat. ↔
tr. preopt intermed., pars ant. ↔
fasc. med. hem. ↔
tr strio-thal. cruc. ↔
nucl. entoped. ↔
tr. preopt entoped
tr strio-thal. incruc. ↔
tr. preopt. intermed., pars lat.
tr. praeth cin.
opticus.

69

PLATE 17

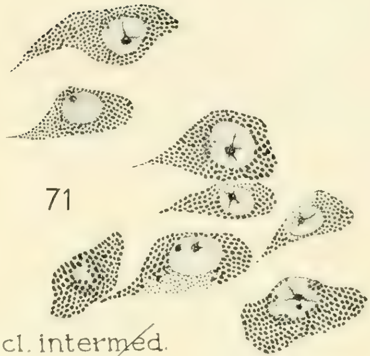
EXPLANATION OF FIGURES

70 Transection through the posterior pole of the hemisphere and the nucleus magnocellularis. Toluidin blue method. × 46.

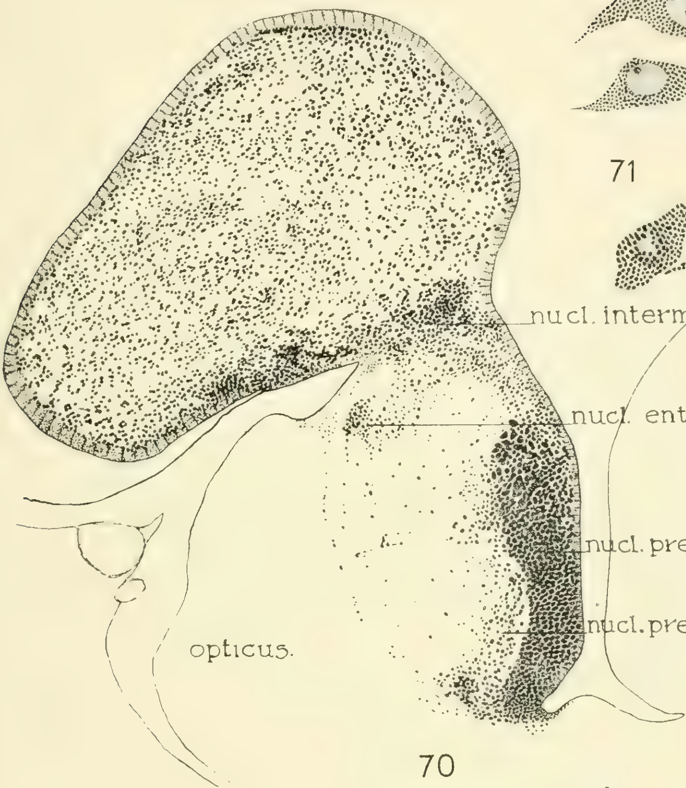
71 A group of cells of the nucleus magnocellularis. Toluidin blue method. × 575.

72 Transection slightly caudal to the level shown in fig. 69. Ramón y Cajal method. × 46.

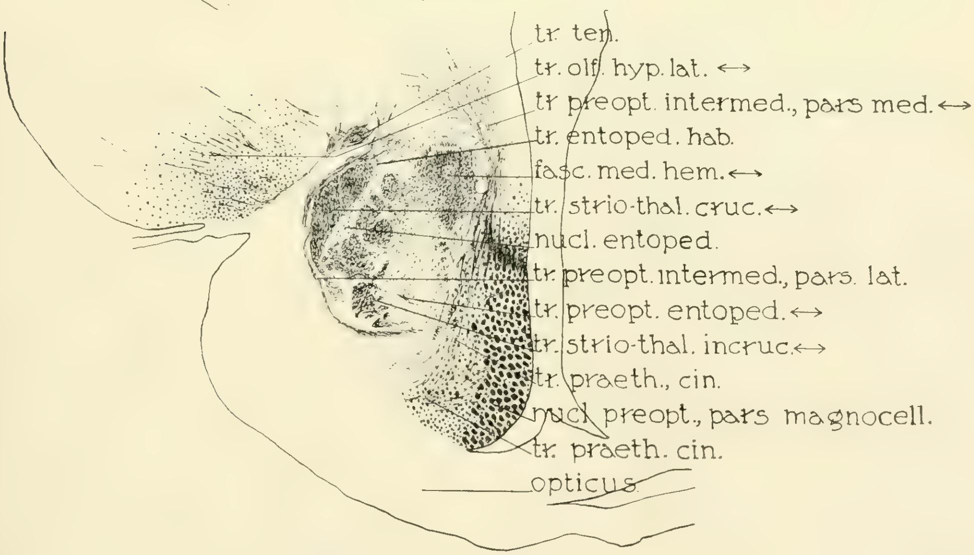
fasc. med. hem. ⟵⟶, fasciculus medialis hemisphaerii; *nucl. entoped.*, nucleus entopeduncularis; *nucl. intermed.*, nucleus intermedius; *nucl. preopt., pars magnocell.*, nucleus preopticus, pars magnocellularis; *nucl. preopt., pars parvocell. lat.*, nucleus preopticus, pars parvocellularis lateralis; *tr. entoped. hab.*, tractus entopedunculo-habenularis; *tr. olf. hyp. lat.* ⟵⟶, tractus olfacto-hypothalamicus lateralis; *tr. praeth. cin.*, tractus praethalamo-cinereus; *tr. preopt. entoped.* ⟵⟶, tractus preoptico-entopeduncularis; *tr. preopt. intermed., pars lat.*, tractus preoptico-intermedius, pars lateralis; *tr. preopt. intermed., pars med.* ⟵⟶, tractus preoptico-intermedius, pars medialis; *tr. strio-thal. cruc.* ⟵⟶, tractus strio-thalamicus cruciatus; *tr. strio-thal. incruc.* ⟵⟶, tractus strio-thalamicus incruciatus; *tr. ten.*, tractus teniae.



71


nucl. intermed.
nucl. entoped.
nucl. preopt., pars magnocell.
nucl. preopt., pars parvocell. lat.
opticus.

70


tr. ten.
tr. olf. hyp. lat. ↔
tr. preopt. intermed., pars med. ↔
tr. entoped. hab.
fasc. med. hem. ↔
tr. strio-thal. cruc. ↔
nucl. entoped.
tr. preopt. intermed., pars. lat.
tr. preopt. entoped. ↔
tr. strio-thal. incruc. ↔
tr. praeth., cin.
nucl. preopt., pars magnocell.
tr. praeth. cin.
opticus.

72

PLATE 18

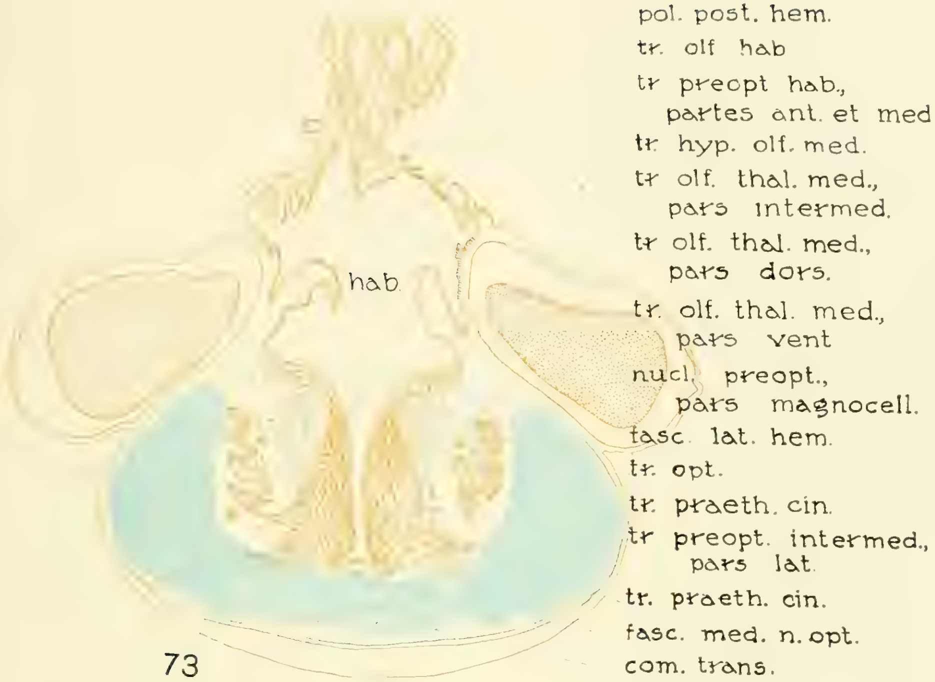
EXPLANATION OF FIGURES

73 Transection at the level of the commissura transversa. Weigert method. × 17.

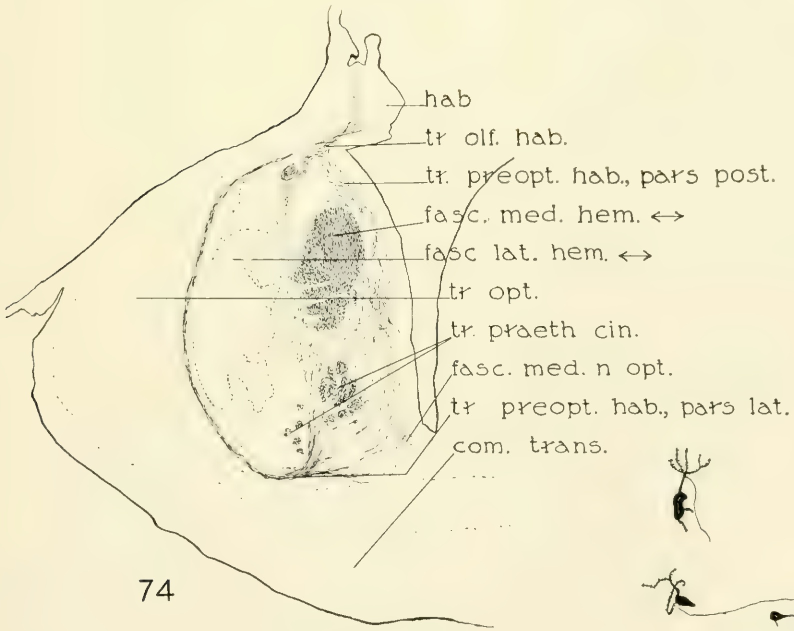
74 Transection through the same region as is shown in fig. 73. Ramón y Cajal method. × 46. Shows particularly the tractus preoptico-habenularis, pars lateralis. In Petromyzon, according to Johnston ('02), a large portion of the fibers of his tractus olfacto-habenularis (my tractus preoptico-habenularis) take this course; as is also the case, but to a lesser extent, in amphibians and reptiles (Herrick, '10 b).

75 Cells of origin of the fasciculus retroflexus, or Meynert's bundle. Golgi method. × 93. One of the cells possesses a long neurite which may be traced into the fasciculus retroflexus.

com. trans., commissura transversa; *fasc. lat. hem.* ←→, fasciculus lateralis hemisphaerii; *fasc. med. hem.* ←→, fasciculus medialis hemisphaerii; *fasc. med. n. opt.*, fasciculus medialis nervi optici; *hab.*, habenula; *nucl. preopt., pars magnocell.*, nucleus preopticus, pars magnocellularis; *pol. post. hem.*, polus posterior hemisphaerii; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. hab.*, tractus olfacto-habenularis; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med., pars intermed.* ←→, tractus olfacto-thalamicus medialis, pars intermedia; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. opt.*, tractus opticus; *tr. praeth. cin.*, tractus praethalamo-cinereus; *tr. preopt. hab., partes ant. et med.*, tractus preoptico-habenularis, partes anterior et medialis; *tr. preopt. hab., pars lat.*, tractus preoptico-habenularis, pars lateralis; *tr. preopt. hab., pars post.*, tractus preoptico-habenularis, pars posterior; *tr. preopt. intermed., pars. lat.*, tractus preoptico-intermedius, pars lateralis.


hab.
pol. post. hem.
tr. olf hab
tr preopt hab.,
partes ant. et med
tr hyp. olf. med.
tr olf. thal. med.,
pars intermed.
tr olf. thal. med.,
pars dors.
tr. olf. thal. med.,
pars vent
nucl. preopt.,
pars magnocell.
fasc. lat. hem.
tr. opt.
tr. praeth. cin.
tr preopt. intermed.,
pars lat.
tr. praeth. cin.
fasc. med. n. opt.
com. trans.

73


hab
tr olf. hab.
tr. preopt. hab., pars post.
fasc. med. hem. ↔
fasc lat. hem. ↔
tr opt.
tr. praeth cin.
fasc. med. n opt.
tr preopt. hab., pars lat.
com. trans.

74

75

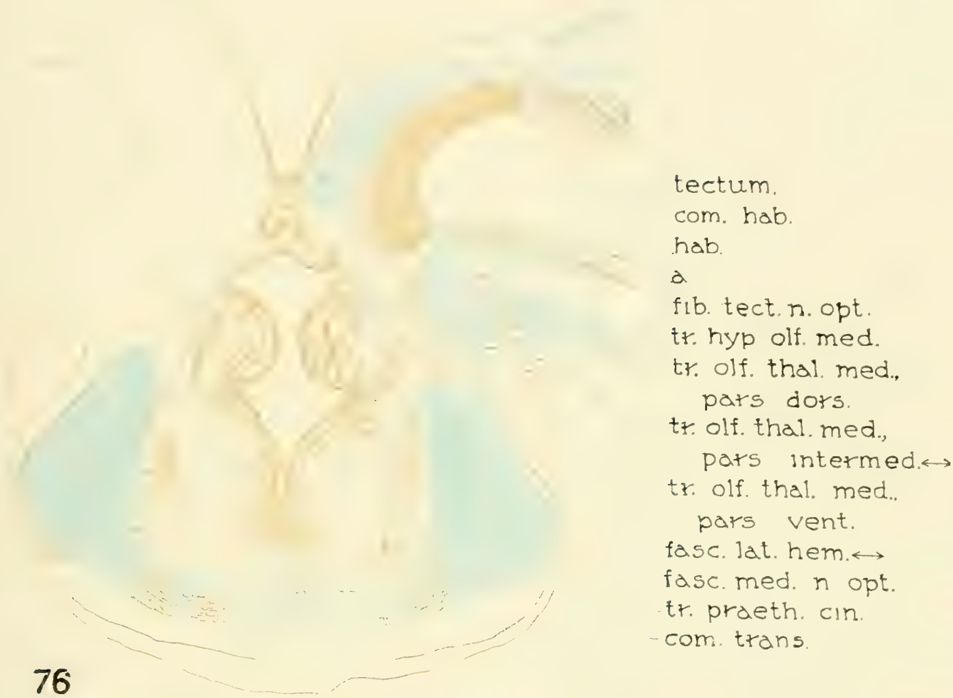
PLATE 19

EXPLANATION OF FIGURES

76 Transection through the ganglia habenularum. Weigert method. × 17.

77 Transection at the level of the commissura horizontalis. Weigert method. × 17.

a, fibers, originating largely in the nucleus posthabenularis and apparently entering the optic tract (described as an optic connection by Bela Haller); *com. hab.*, commissura habenularis, containing also decussating fibers of the two tractus olfacto-habenulares; *com. Herrick*, commissura Herricki; *com. horiz.*, commissura horizontalis of Fritsch; *com. trans.*, commissura transversa; *corp. gen. lat.*, corpus geniculatum laterale; *fasc. lat. hem.* ←→, fasciculus lateralis hemisphaerii; *fasc. med. n. opt.*, fasciculus medialis nervi optici; *fasc. retr.*, fasciculus retroflexus; *fib. tect. n. opt.*, fibrae tectales nervi optici (centrifugal); *hab.*, habenula; *lob. inf.*, lobus inferior; *nucl. preopt., pars parvocell. post.*, nucleus preopticus. pars parvocellularis posterior; *nucl. prerot.*, nucleus prerotundus; *nucl. vent. tub.*, nucleus ventralis tuberis; *tr. hab. dien .* ←→, tractus habenulo-diencephalicus; this is the 'tractus habenula ad diencephalon' of Goldstein; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. hab.*, tractus olfacto-habenularis; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med., pars intermed.* ←→, tractus olfacto-thalamicus medialis, pars intermedia; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. opt.*, tractus opticus; *tr. praeth. cin.*, tractus praethalamo-cinereus.


tectum.
com. hab.
hab.
a
fib. tect. n. opt.
tr. hyp olf. med.
tr. olf. thal. med., pars dors.
tr. olf. thal. med., pars intermed.↔
tr. olf. thal. med., pars vent.
fasc. lat. hem.↔
fasc. med. n opt.
tr. praeth. cin.
com. trans.

76


corp. gen. lat.
hab.
fasc. retr.
fib. tect. n. opt.
tr. olf. hab.
a.
tr. hab. dien. ↔
tr. hyp. olf. med.
tr. opt.
tr. olf. thal. med., pars dors
tr. olf. thal. med., pars intermed ↔
tr. olf. thal. med., pars vent.
fasc. lat. hem.↔
nucl. preopt., pars parvocell. post.
fasc. med. n. opt.
com. Herrick.
tr. praeth. cin.
com. trans.
com. horiz.
lob. inf.
nucl. prerot.
nucl. vent. tub.

77

PLATE 20

EXPLANATION OF FIGURES

78 Transection through the ganglia habenularum. Toluidin blue method. × 46.

com. horiz., commissura horizontalis; *corp. gen. lat.*, corpus geniculatum laterale; *fib. ans.*, fibrae ansulatae; *hab.*, habenula, showing characteristic arrangement of cells; *hyp.*, hypophysis; *lob. inf.*, lobus inferior; *nucl. ant. thal.*, nucleus anterior thalami of Goldstein; *nucl. posthab.*, nucleus posthabenularis, 'Das posthabenulare Zwischenhirngebiet' of Goldstein, 'Die posthabenulare Zwischenhirngegend' of Bela Haller; *nucl. preopt., pars parvocell. post.*, nucleus preopticus. pars parvocellularis posterior; *nucl. prerot.*, nucleus prerotundus; *nucl. vent. tub.*, nucleus ventralis tuberis.

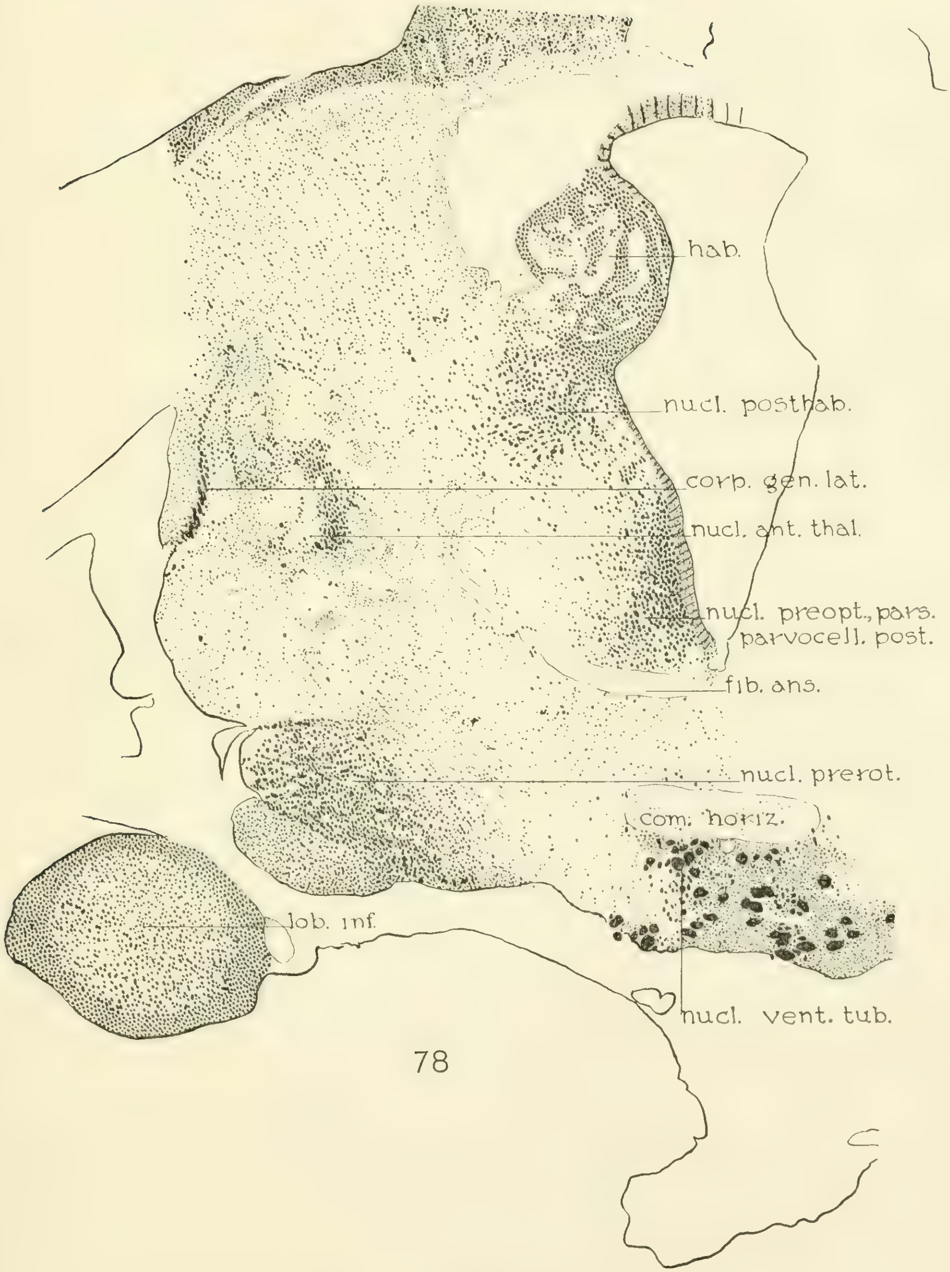

hab.
nucl. posthab.
corp. gen. lat.
nucl. ant. thal.
nucl. preopt., pars. parvocell. post.
fib. ans.
nucl. prerot.
com. horiz.
lob. inf.
nucl. vent. tub.
78

PLATE 21

EXPLANATION OF FIGURES

79 Transection slightly caudal to the level shown in fig. 77. Weigert method. × 17.

80 Transection through the nucleus anterior tuberis. Weigert method. × 17.

a, see fig. 76; *com. Herrick.*, commissura Herricki; *com. horiz.*, commissura horizontalis; *com. post.*, commissura posterior; *com. trans.*, commissura transversa; *corp. gen. lat.*, corpus geniculatum laterale; *fasc. lat. hem.*⟵⟶, fasciculus lateralis hemisphaerii; *fasc. med. n. opt.*, fasciculus medialis nervi optici; *fasc. retr.*, fasciculus retroflexus; *fib. ans.*, fibrae ansulatae; *fib. tect. n. opt.*, fibrae tectales nervi optici; *hab.*, habenula; *hyp.*, hypophysis; *lob. inf.*, lobus inferior; *nucl. ant. thal.*, nucleus anterior thalami; *nucl. ant. tub.*, nucleus anterior tuberis; *nucl. lat. tub.*, nucleus lateralis tuberis; *nucl. prerot.*, nucleus prerotundus; *nucl. vent. tub.*, nucleus ventralis tuberis; *tectum*, tectum opticum; *tor. long.*, torus longitudinalis; *tr. hab. dien.*⟵⟶, tractus habenulo-diencephalicus; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med., pars intermed.*⟵⟶, tractus olfacto-thalamicus medialis, pars intermedia; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. opt.* tractus opticus; *tr. praeth. cin.*, tractus praethalamo-cinereus; *tr. strio-thal. incruc.*⟵⟶, tractus strio-thalamicus incruciatus; *tr. tub. dors.*, tractus tubero-dorsalis; *valvula*, valvula cerebelli.


corp. gen. lat.
fib. tect. n. opt.
hab.
fasc. retr.
a
tr. hab. dien.↔
tr. hyp. olf. med.
tr. olf. thal. med., pars intermed.↔
tr. olf. thal. med., pars dors
tr. olf. thal. med., pars vent
tr. opt.
fasc. lat. hem.↔
com. Herrick.
fasc. med. n. opt.
tr. praeth. cin.
com. trans.
com. horiz.
lob. inf.
nucl prerot.
nucl. vent. tub.

79


com. post.
fib. tect. n. opt.
hab
fasc. retr.
nucl. ant. thal.
corp. gen. lat.
tr. opt.
tr. hab. dien.↔
tr. hyp. olf. med
tr. olf. thal. med., pars dors.
tr. olf. thal. med., pars intermed..↔
tr. olf. thal. med., pars vent.
com. trans.
fib. ans.
lob. inf.
nucl. prerot.
tr. strio-thal. incruc.↔
com. horiz.
tr. tub. dors
nucl. ant. tub.
tr. praeth. cin.
nucl. lat. tub.
nucl. vent. tub.
hyp.

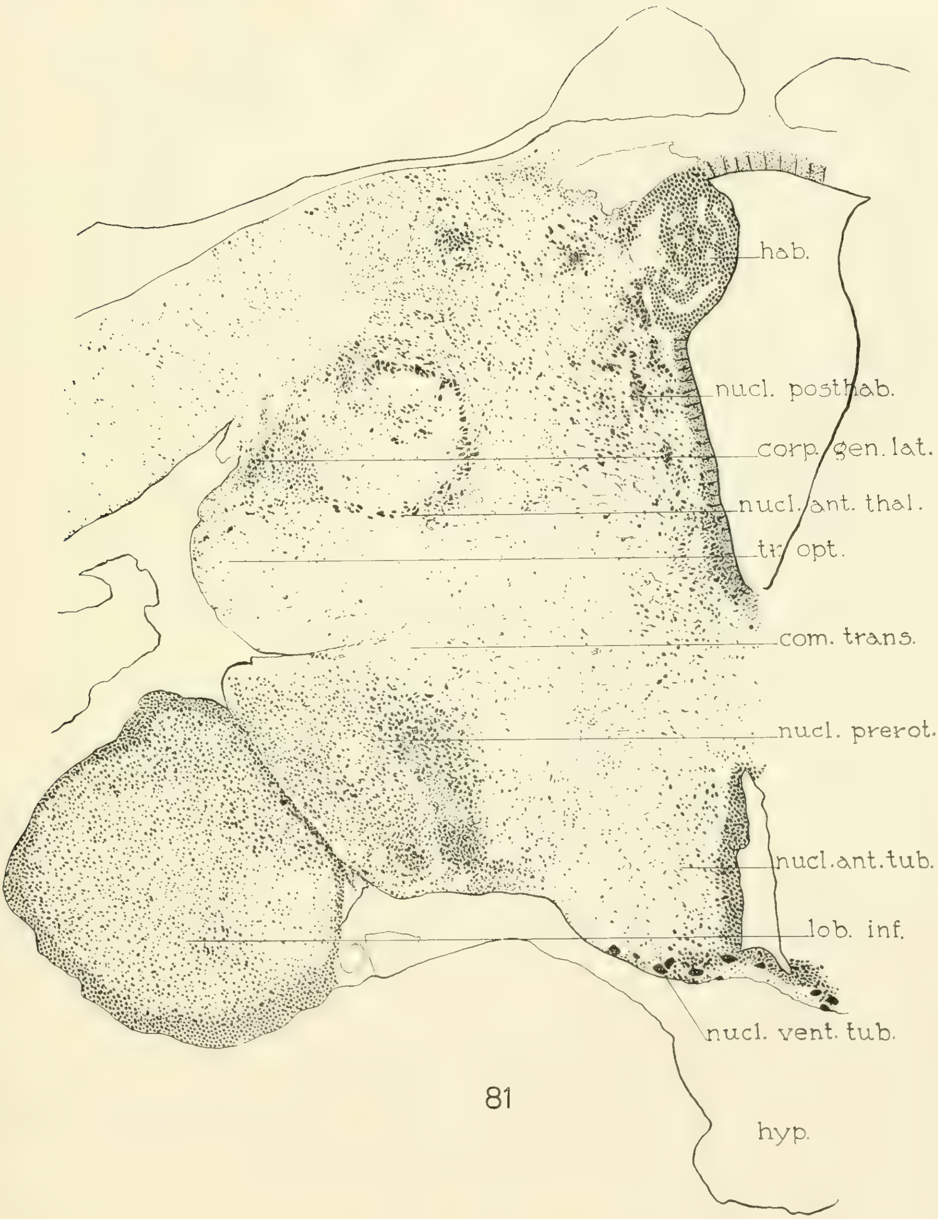
80

PLATE 22

EXPLANATION OF FIGURES

81 Transection through the nucleus anterior tuberis. Toluidin blue method. × 46.

com. trans., commissura transversa; *corp. gen. lat.*, corpus geniculatum laterale; *hab.*, habenula; *hyp.*, hypophysis; *lob. inf.*, lobus inferior; *nucl. ant. thal.*, nucleus anterior thalami; *nucl. ant. tub.*, nucleus anterior tuberis; *nucl. posthab.*, nucleus posthabenularis; *nucl. prerot.*, nucleus prerotundus; *nucl. vent. tub.*, nucleus ventralis tuberis; *tr. opt.*, tractus opticus.


hab.
nucl. posthab.
corp. gen. lat.
nucl. ant. thal.
tr. opt.
com. trans.
nucl. prerot.
nucl. ant. tub.
lob. inf.
nucl. vent. tub.
hyp.

81

PLATE 23

EXPLANATION OF FIGURES

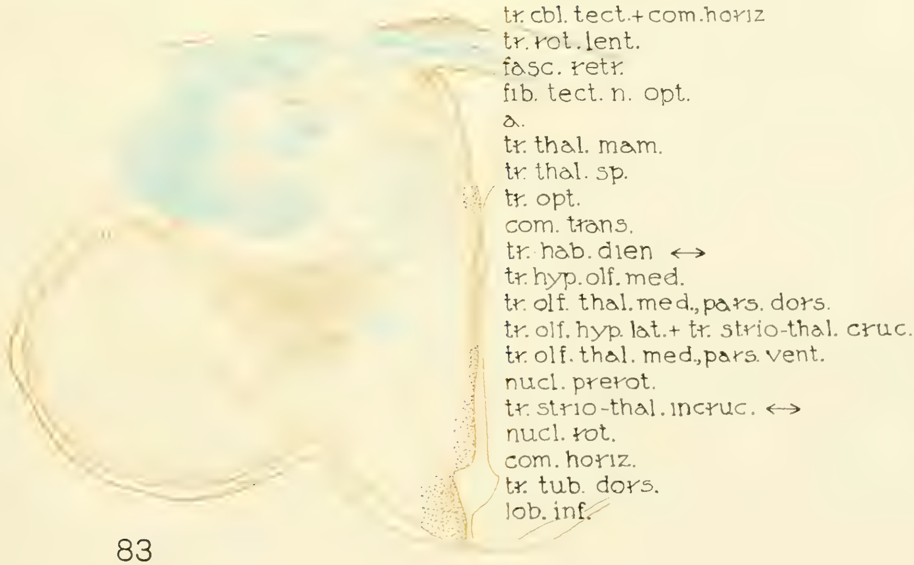
82 Transection slightly caudal to the level shown in fig. 80. Weigert method. × 17.

83 Transection through the rostral end of the nucleus rotundus. Weigert method. × 17. Shows particularly the connections between the fasciculus lateralis hemisphaerii and the nuclei prerotundus, rotundus and diffusus lobi lateralis.

a, (see fig. 76); *corp. gen. lat.*, corpus geniculatum laterale; *com. horiz.*, commissura horizontalis; *com. post.*, commissura posterior; *com. trans.*, commissura transversa; *fasc. retr.*, fasciculus retroflexus; *fib. ans.*, fibrae ansulatae; *fib. tect. n. opt.*, fibrae tectales nervi optici; *hyp.* hypophysis; *lob. inf.*, lobus inferior; *nucl. ant. thal.*, nucleus anterior thalami; *nucl. ant. tub.*, nucleus anterior tuberis; *nucl. lat. tub.*, nucleus lateralis tuberis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *nucl. vent. tub.*, nucleus ventralis tuberis; *tr. cbl. tect. + com. horiz.*, tractus cerebello-tectalis plus the commissura horizontalis; *tr. hab. dien.*⟵⟶, tractus habenulo-diencephalicus; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. hyp. lat. + tr. strio-thal. cruc.*, tractus olfacto-hypothalamicus lateralis plus the tractus strio-thalamicus cruciatus; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med., pars intermed.*⟵⟶, tractus olfacto-thalamicus medialis, pars intermedia; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. opt.*, tractus opticus; *tr. praeth. cin.*, tractus praethalamo-cinereus; *tr. rot. lent.*, tractus rotundo-lentiformis (Kappers); *tr. strio-thal. incruc.*⟵⟶, tractus strio-thalamicus incruciatus; shows particularly the fibers innervating the nucleus diffusus lobi lateralis; *tr. thal. mam.*, tractus thalamo-mammillaris (Goldstein); *tr. thal. sp.*, tractus thalamo-spinalis (Kappers); *tr. tub. dors.*, tractus tubero-dorsalis.


com. post.
fasc. retr.
nucl. ant. thal.
corp. gen. lat.
tr. hab. dien. ⟷
tr. hyp. olf. med.
tr. olf. thal. med., pars. intermed. ⟷
tr. olf. thal. med., pars. dors.
tr. opt.
tr. olf. thal. med., pars. vent.
com. trans.
fib. ans.
tr. strio-thal. incruc. ⟷
com. horiz.
nucl. prerot.
tr. tub. dors.
nucl. ant. tub.
tr. strio-thal. incruc. ⟷
nucl. lat. tub.
tr. praeth. cin.
nucl. vent. tub.
lob. inf.
hyp.

82


tr. cbl. tect. + com. horiz
tr. rot. lent.
fasc. retr.
fib. tect. n. opt.
a.
tr. thal. mam.
tr. thal. sp.
tr. opt.
com. trans.
tr. hab. dien ⟷
tr. hyp. olf. med.
tr. olf. thal. med., pars. dors.
tr. olf. hyp. lat. + tr. strio-thal. cruc.
tr. olf. thal. med., pars. vent.
nucl. prerot.
tr. strio-thal. incruc. ⟷
nucl. rot.
com. horiz.
tr. tub. dors.
lob. inf.

83

PLATE 24

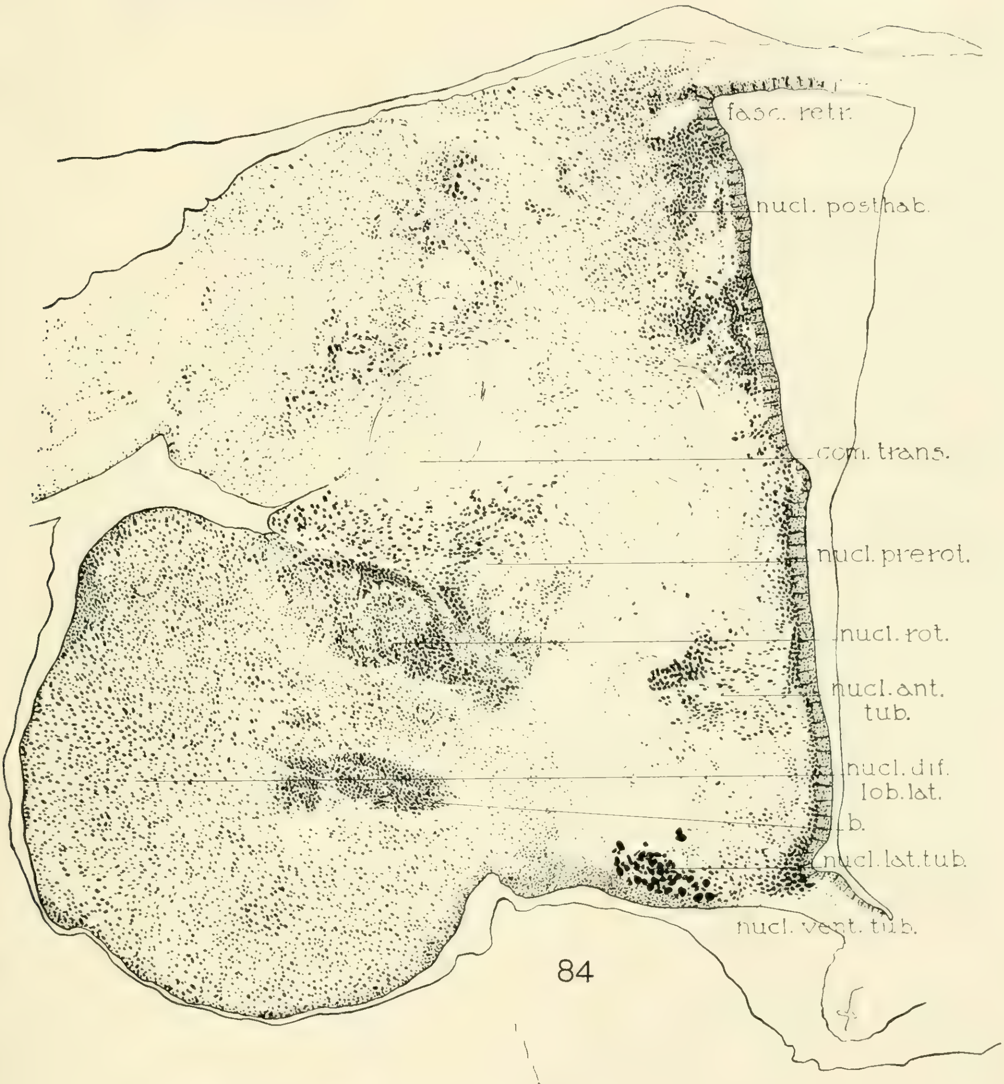
EXPLANATION OF FIGURES

84 Transection through the rostral end of the nucleus rotundus. Toluidin blue method. × 46.

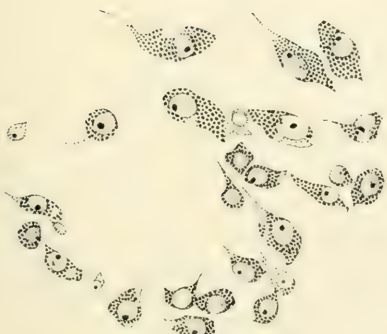
85 Cells of the nucleus prerotundus. Toluidin blue method. × 575. From the right nucleus. Shows the scattered arrangement of the cells and their difference in size.

86 Neurones of the nucleus prerotundus. Golgi method. ×93. From sagittal section.

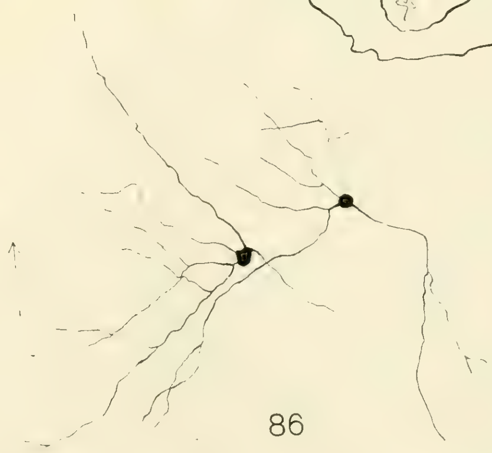
b, cells adjacent to the recessus lateralis hypothalami (see fig. 87); *com. trans.*, commissura transversa; *fasc. retr.*, fasciculus retroflexus; *nucl. ant. tub.*, nucleus anterior tuberis; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. lat. tub.*, nucleus lateralis tuberis; *nucl. posthab.*, nucleus posthabenularis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus ; *nucl. vent. tub.*, nucleus ventralis tuberis.


fasc. retr.
nucl. post hab.
com. trans.
nucl. prerot.
nucl. rot.
nucl. ant. tub.
nucl. dif. lob. lat.
l.b.
nucl. lat. tub.
nucl. vent. tub.

84



85



86

PLATE 25

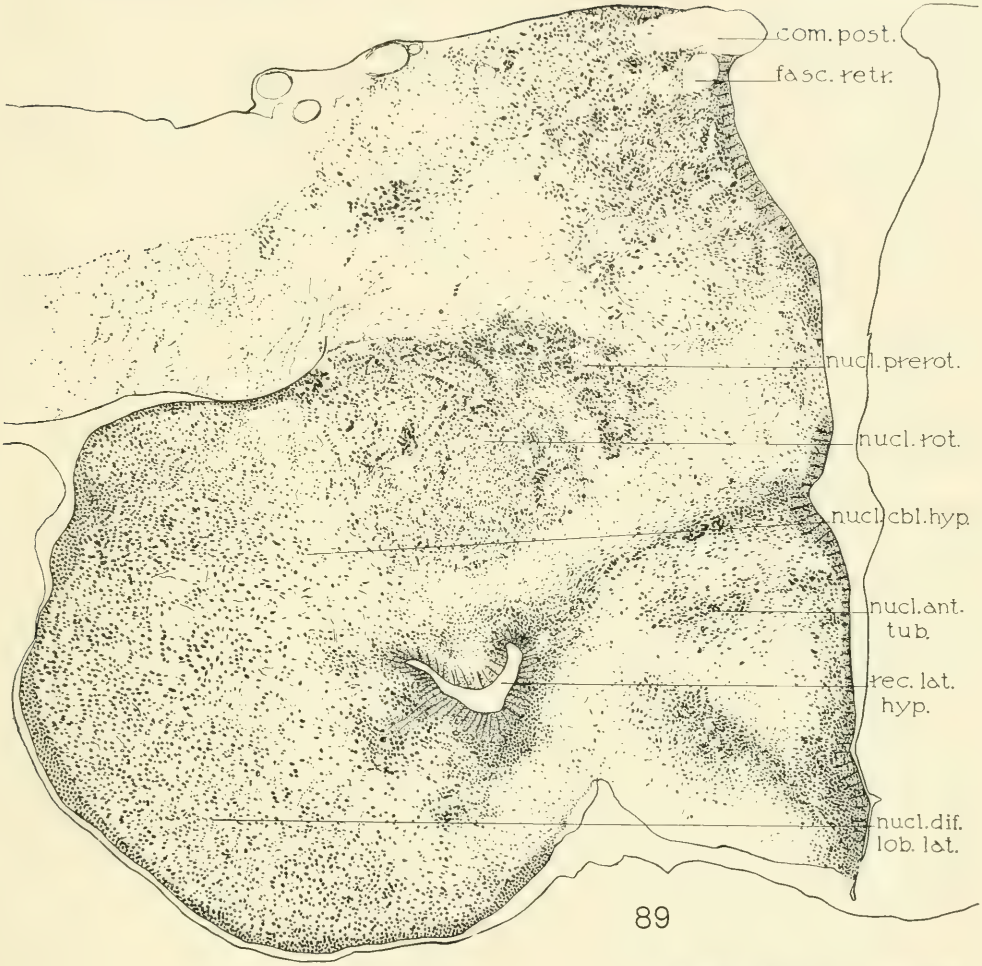
EXPLANATION OF FIGURES

87–88 Neurones of the nucleus prerotundus, Golgi method. × 93. From sagittal sections.

89 Transection immediately caudal to the level of the commissura posterior. Toluidin blue method. × 46.

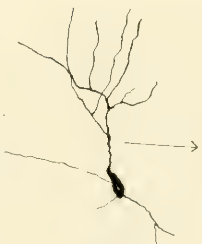
90 Cells of the nucleus rotundus. Toluidin blue method. × 575. Shows the typical arrangement of the cells in scattered groups.

com. post., commissura posterior; *fasc. retr.*, fasciculus retroflexus; *nucl. ant. tub.*, nucleus anterior tuberis; *nucl. cbl. hyp.*, nucleus cerebellaris hypothalami; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *rec. lat. hyp.*, recessus lateralis hypothalami.

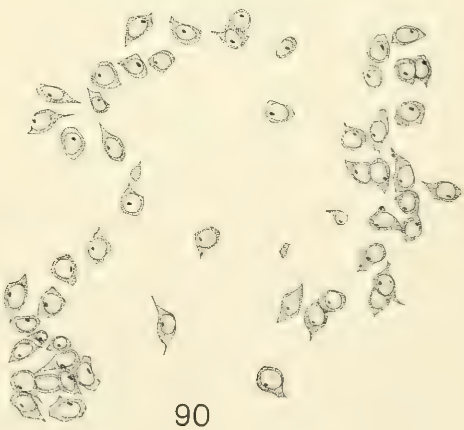

com. post.
fasc. retr.
nucl. prerot.
nucl. rot.
nucl. cbl. hyp.
nucl. ant. tub.
rec. lat. hyp.
nucl. dif. lob. lat.

89

87



88



90

PLATE 26

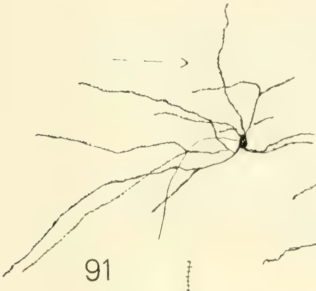
EXPLANATION OF FIGURES

91–94 Neurones of the nucleus rotundus. Golgi method. × 93. From sagittal sections.

95–99 Neurones from different parts of the lobi laterales. Golgi method. × 93. From sagittal sections. Fig. 95 is taken from the caudal angle of the lobe, fig. 96 from the ventro-medial area, fig. 97 from the latero-medial, figs. 98 and 99 from the ventral area proper.

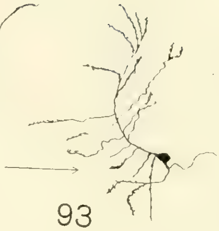
100 Transection at approximately the same level as that shown in fig. 89. Weigert method. × 17.

com. horiz., commissura horizontalis; *com. post.*, commissura posterior; *com. trans.*, commissura transversa; *fasc. retr.*, fasciculus retroflexus; *nucl. ant. tub.*, nucleus anterior tuberis; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *tr. cbl. tect. + com. horiz.*, tractus cerebello-tectalis plus commissura horizontalis; *tr. hab. dien.* ←→, tractus habenulo-diencephalicus; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. rot. lent.*, tractus rotundo-lentiformis; *tr. strio-thal. cruc. + tr. olf. hyp. lat.*, tractus strio-thalamicus cruciatus plus tractus olfacto-hypothalamicus lateralis; *tr. thal. mam.*, tractus thalamo-mammillaris; *tr. thal. sp.*, tractus thalamo-spinalis.

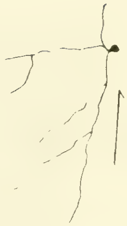


91

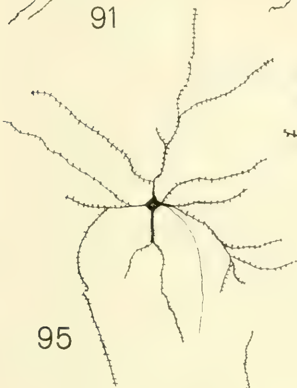
92



93

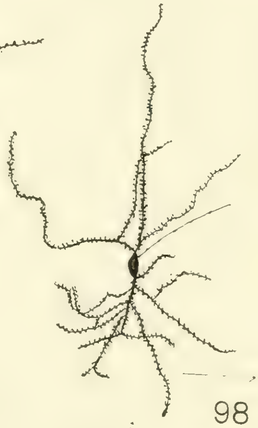


94

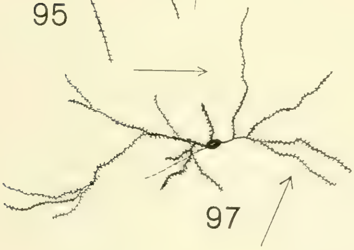


95

96



98



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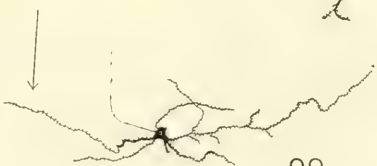


99

100

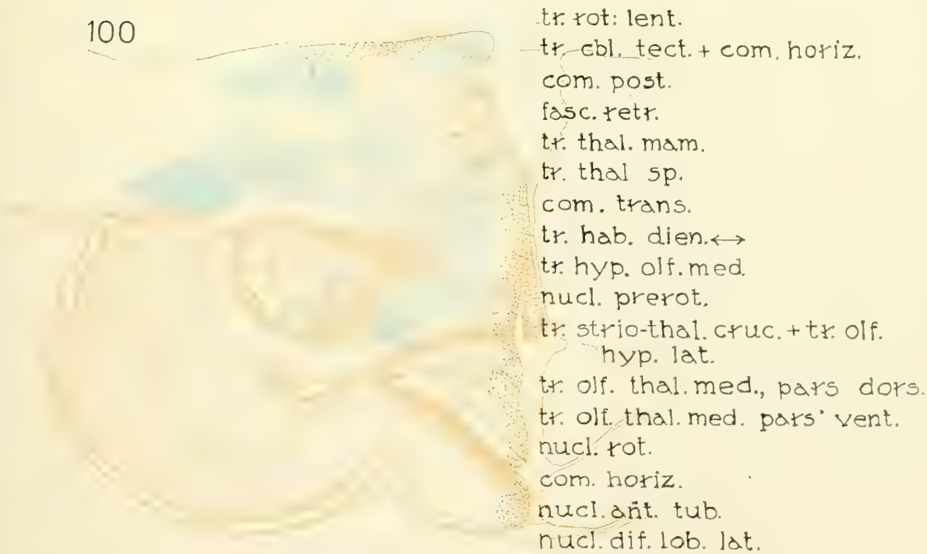


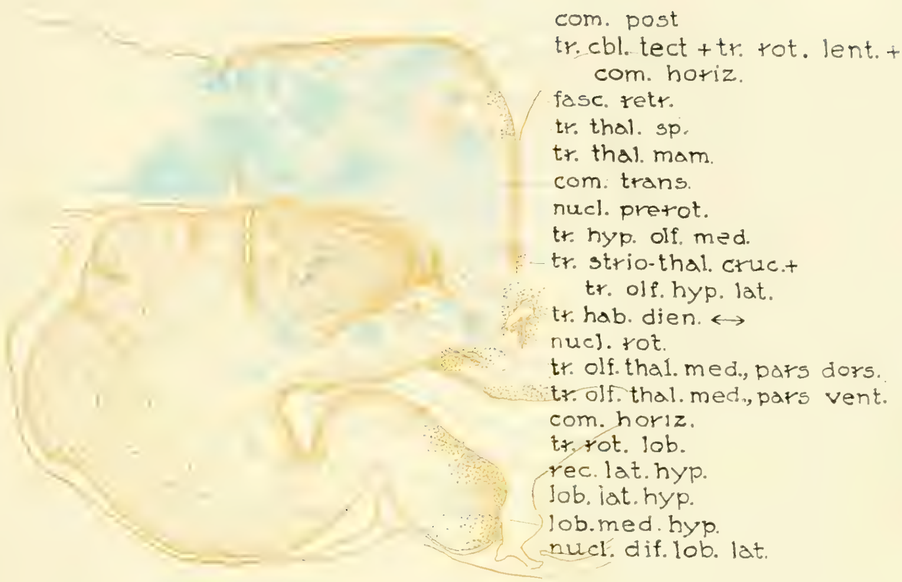
PLATE 27

EXPLANATION OF FIGURES

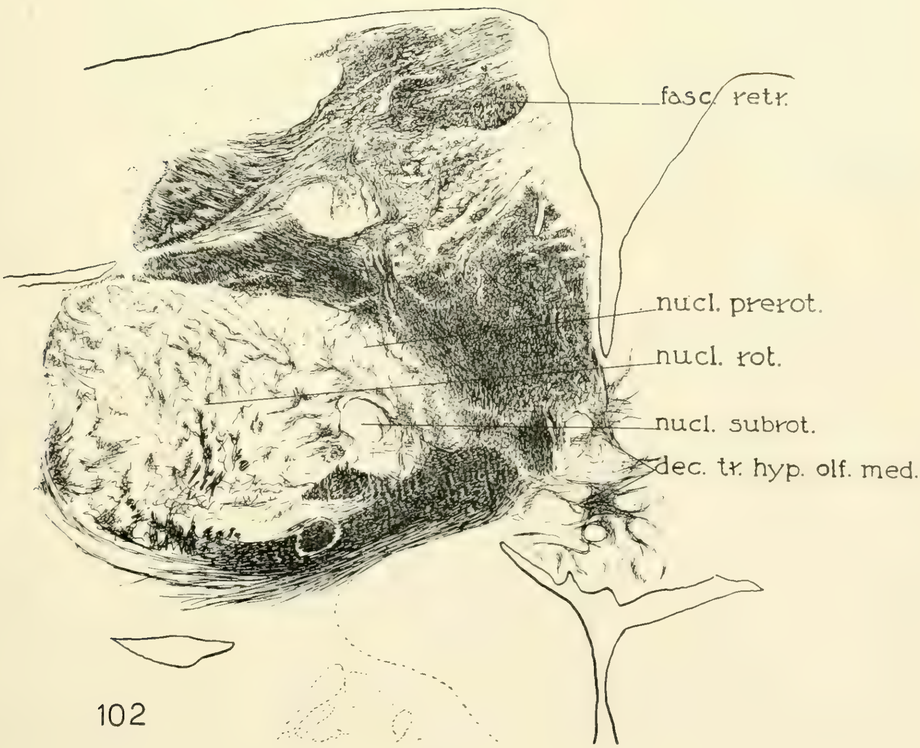
101 Transection slightly caudal to the level shown in fig. 100. Weigert method. × 17.

102 Transection through the decussation of the tractus hypothalamo-olfactorii mediales. Ramón y Cajal method. × 46.

com. horiz., commissura horizontalis; *com. post.*, commissura posterior; *com. trans.*, commissura transversa; *dec. tr. hyp. olf. med.*, decussatio tractorum olfactoriorum medialium; *fasc. retr.* fasciculus retroflexus; *lob. lat. hyp.*, lobus lateralis hypothalami; *lob. med. hyp.*, lobus medialis hypothalami; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *nucl. subrot.*, nucleus subrotundus; *rec. lat. hyp.*, recessus lateralis hypothalami; *tr. cbl. tect.* + *tr. rot. lent.* + *com. horiz.*, tractus cerebellotectalis plus tractus rotundo-lentiformis plus commissura horizontalis; *tr. hab. dien.*←→, tractus habenulo-diencephalicus; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. olf. thal. med.*, *pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med.*, *pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. rot. lob.*, tractus rotundo-lobaris; *tr. strio-thal. cruc.* + *tr. olf. hyp. lat.*, tractus strio-thalamicus cruciatus plus tractus olfacto-hypothalamicus lateralis; *tr. thal. mam.*, tractus thalamo-mammillaris; *tr. thal. sp.*, tractus thalamo-spinalis (Kappers).


com. post
tr. cbl. tect + tr. rot. lent. + com. horiz.
fasc. retr.
tr. thal. sp.
tr. thal. mam.
com. trans.
nucl. prerot.
tr. hyp. olf. med.
tr. strio-thal. cruc. + tr. olf. hyp. lat.
tr. hab. dien. ↔
nucl. rot.
tr. olf. thal. med., pars dors.
tr. olf. thal. med., pars vent.
com. horiz.
tr. rot. lob.
rec. lat. hyp.
lob. lat. hyp.
lob. med. hyp.
nucl. dif. lob. lat.

101


fasc. retr.
nucl. prerot.
nucl. rot.
nucl. subrot.
dec. tr. hyp. olf. med.

102

PLATE 28

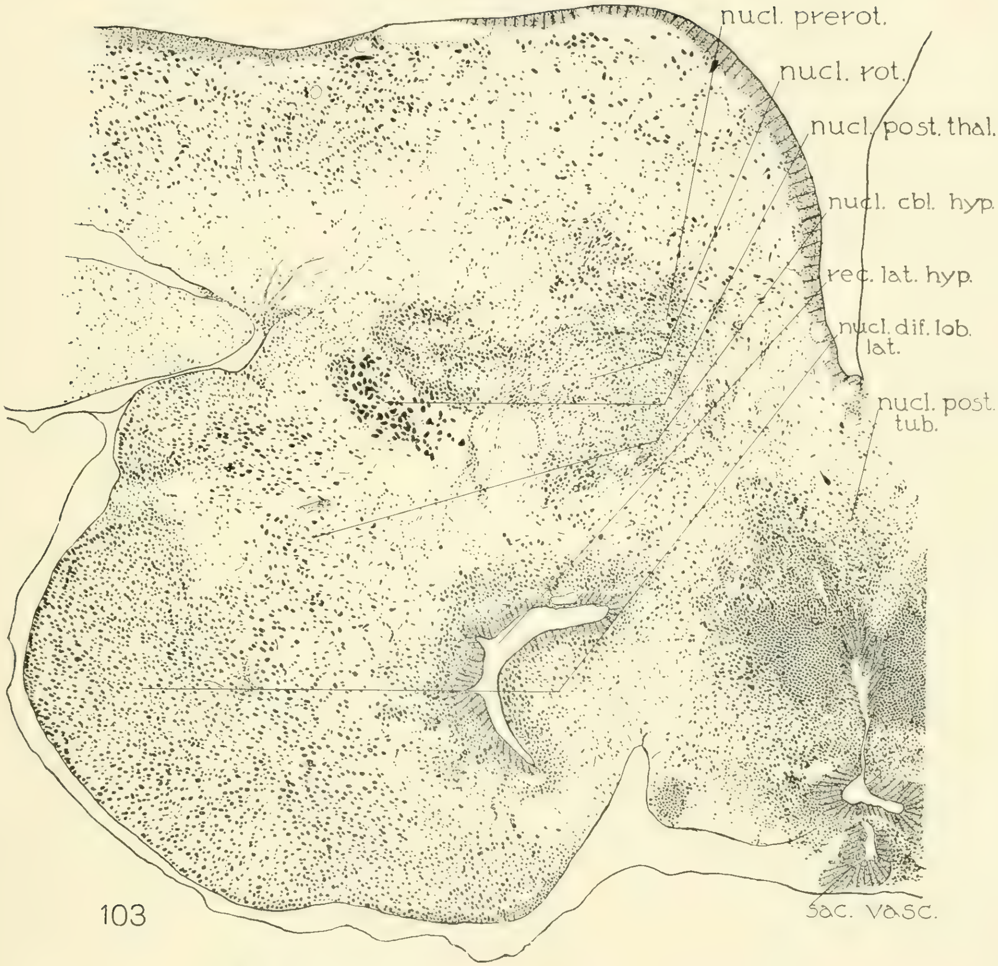
EXPLANATION OF FIGURES

103 Transection through the nucleus posterior tuberis. Toluidin blue method. × 46.

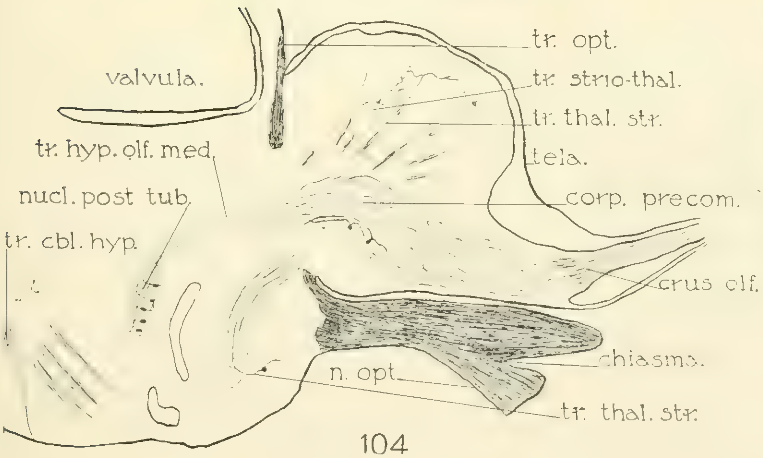
104 Oblique longitudinal section showing the origin of the tractus hypothalamo-olfactorius medialis. Golgi method. × 9.

105 Neurone of origin of the tractus hypothalamo-olfactorius medialis. Golgi method. × 93. Taken from same section as fig. 104.

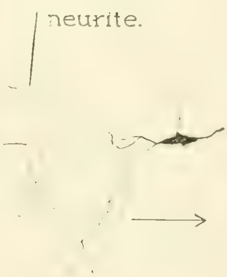
chiasma, optic chiasma; *corp. precom.*, corpus precommissurale; *crus olf.*, crus olfactorium; *n. opt.*, nervus opticus; *nucl. cbl. hyp.*, nucleus cerebellaris hypothalami; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. post. thal.*, nucleus posterior thalami; *nucl. post. tub.*, nucleus posterior tuberis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *rec. lat. hyp.*, recessus lateralis hypothalami; *sac. vasc.*, saccus vasculosus; *tela*, membranous roof of forebrain; *tr. cbl. hyp.*, tractus cerebello-hypothalamicus; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. opt.*, tractus opticus; *tr. strio-thal.*, tractus striothalamicus; *tr. thal. str.*, tractus thalamo-striaticus; *valvula*, valvula cerebelli.


nucl. prerot.
nucl. rot.
nucl. post. thal.
nucl. cbl. hyp.
rec. lat. hyp.
nucl. dif. lob. lat.
nucl. post. tub.
sac. vasc.

103


valvula.
tr. opt.
tr. strio-thal.
tr. thal. str.
tela.
corp. precom.
tr. hyp. olf. med.
nucl. post tub.
tr. cbl. hyp.
crus olf.
chiasma.
n. opt.
tr. thal. str.

104


neurite.

105

PLATE 29

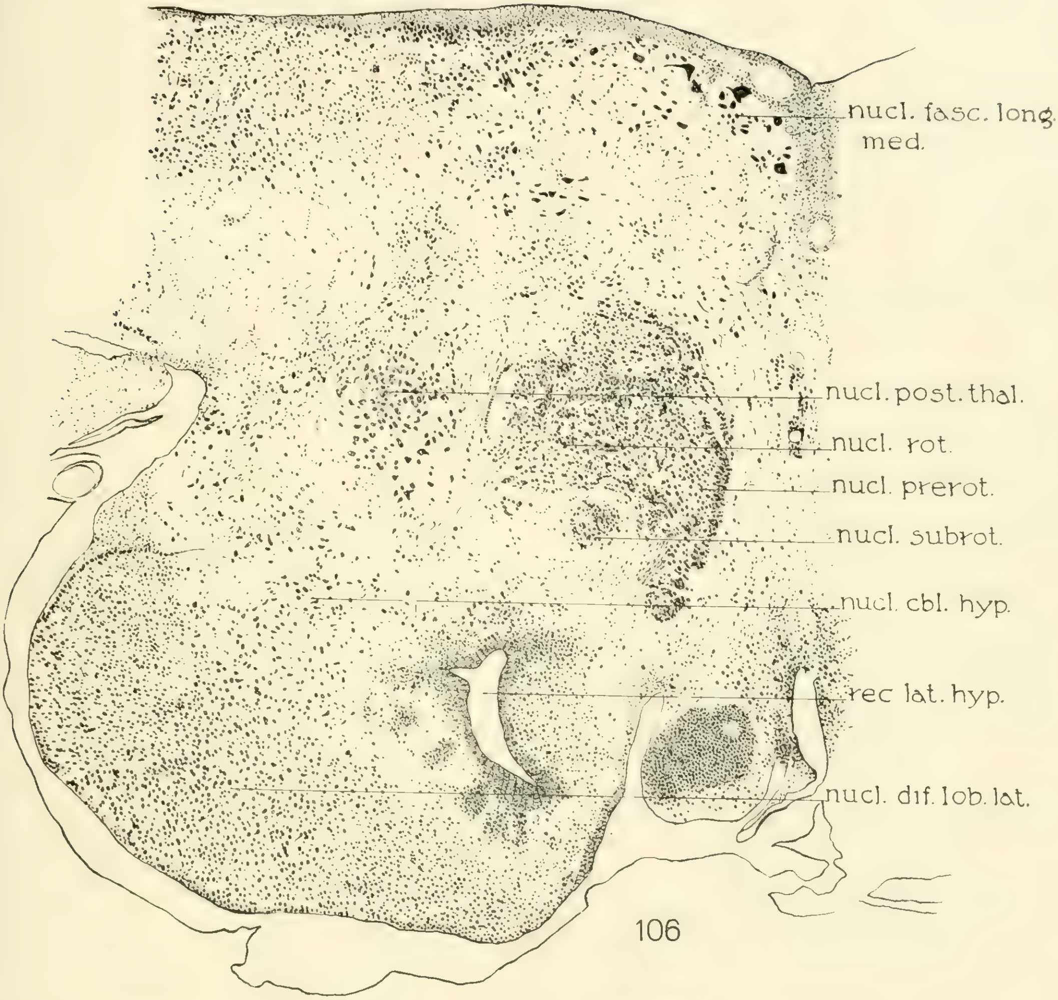
EXPLANATION OF FIGURES

106 Transection through the Haubenwulst. Toluidin blue method. × 46.

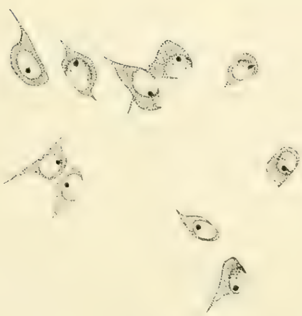
107 Transection through the nucleus subrotundus. Toluidin blue method. × 47. Detail cells. × 575. Shows the characteristic grouping of the cells in the center of the nucleus (see fig. 106). From right side.

108 Cells of the nucleus cerebellaris hypothalami. Toluidin blue method. × 575. Shows typical scattered arrangement of the cells (see figs. 103 and 106).

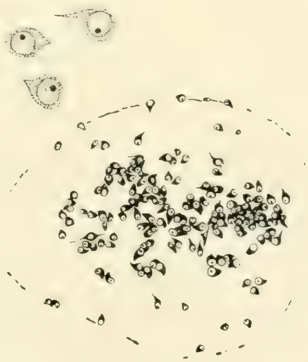
nucl. cbl. hyp., nucleus cerebellaris hypothalami; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. fasc. long. med.*, nucleus fasciculi longitudinalis medialis; *nucl. post. thal.*, nucleus posterior thalami; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *nucl. subrot.* nucleus subrotundus; *rec. lat. hyp.*, recessus lateralis hypothalami.


nucl. fasc. long. med.
nucl. post. thal.
nucl. rot.
nucl. prerot.
nucl. subrot.
nucl. cbl. hyp.
rec lat. hyp.
nucl. dif. lob. lat.

106



108



107

PLATE 30

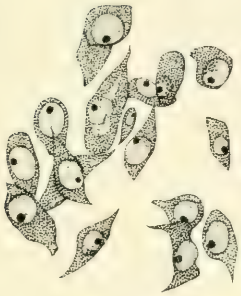
EXPLANATION OF FIGURES

109 Cells of the nucleus posterior thalami. Toluidin blue method. × 575.

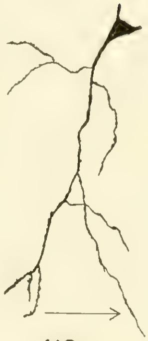
110–113 Neurones of the nucleus posterior thalami. Golgi method. × 93. From sagittal sections. As will be noted, the cells of this nucleus are very large. They appear larger in sagittal than in transverse sections, both in toluidin blue and Golgi material.

114 Transection at approximately the same level as that shown in fig. 106. Weigert method. × 17.

com. horiz., commissura horizontalis; *com. post.*, commissura posterior; *com. trans.*, commissura transversa; *fasc. retr.*, fasciculus retroflexus; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. post. tub.*, nucleus posterior tuberis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *nucl. subrot.*, nucleus subrotundus; *sac. vasc.*, saccus vasculosus; *tr. cbl. tect. + tr. rot. lent. + com. horiz.*, tractus cerebello-tectalis plus tractus rotundo-lentiformis plus commissura horizontalis; *tr. cbl. hyp.*, tractus cerebello-hypothalamicus; *tr. olf. thal. med., pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med., pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. rot. lob.*, tractus rotundo-lobaris; *tr. thal. mam.*, tractus thalamo-mammillaris; *tr. thal. sp.*, tractus thalamo-spinalis (Kappers).

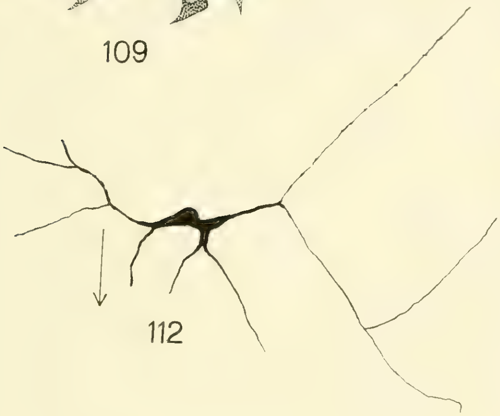


109

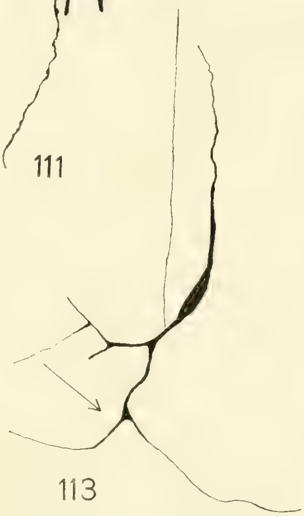


110

111



112



113

114

PLATE 31

EXPLANATION OF FIGURES

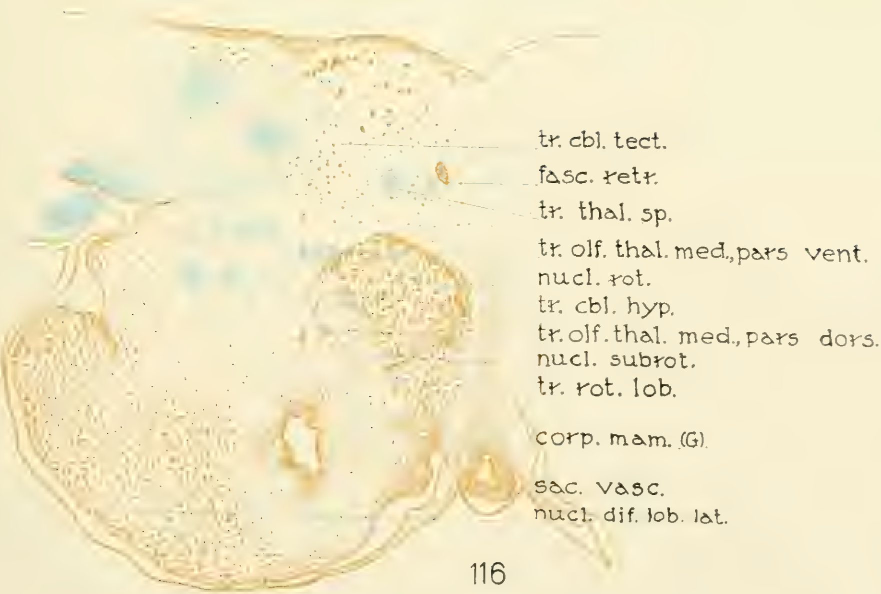
115 Transection slightly caudal to the level shown in fig. 114. Weigert method. × 17.

116 Transection slightly caudal to the level shown in fig. 115. Weigert method. × 17.

com. horiz. + tr. rot. lent., commissura horizontalis plus tractus rotundo-lentiformis; *com. trans.*, commissura transversa; *corp. mam.* (*G*), corpus mammillare, the ganglion mammillare of Goldstein; *fasc. retr.*, fasciculus retroflexus; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. post. thal.*, nucleus posterior thalami; *nucl. post. tub.*, nucleus posterior tuberis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *nucl. subrot.*, nucleus subrotundus; *sac. vasc.*, saccus vasculosus; *tr. cbl. tect.*, tractus cerebello-tectalis; *tr. cbl. tect. + tr. rot. lent. + com. horiz.*, tractus cerebello-tectalis plus tractus rotundo-lentiformis plus commissura horizontalis; *tr. cbl. hyp.*, tractus cerebello-hypothalamicus; *tr. olf. thal. med., pars. dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; its connection with the nucleus subrotundus comes out with great clearness in these figures; *tr. olf. thal. med., pars. vent.*, tractus olfacto-thalamicus medialis, pars ventralis; in fig. 116, immediately lateral to the nucleus rotundus, is shown the termination of this tract; *tr. rot. lob.*, tractus rotundo-lobaris; *tr. thal. mam.*, tractus thalamo-mammillaris; *tr. thal. sp.*, tractus thalamo-spinalis (Kappers.)


tr. cbl. tect + tr. rot. lent. + com. horiz.
tr. thal. sp.
com. trans.
tr. thal. mam.
com. horiz. + tr. rot. lent.
nucl. post. thal.
nucl. prerot.
tr. olf. thal. med., pars vent.
nucl. rot.
tr. olf. thal. med., pars dors.
tr. rot. lob.
nucl. subrot.
nucl. post. tub.
tr. cbl. hyp.
nucl. dif. lob. lat.
sac. vasc.

115


tr. cbl. tect.
fasc. retr.
tr. thal. sp.
tr. olf. thal. med., pars vent.
nucl. rot.
tr. cbl. hyp.
tr. olf. thal. med., pars dors.
nucl. subrot.
tr. rot. lob.
corp. mam. (G).
sac. vasc.
nucl. dif. lob. lat.

116

PLATE 32

EXPLANATION OF FIGURES

117 Transection through the corpus mammillare, the ganglion mammillare of Goldstein. Toluidin blue method. × 46.

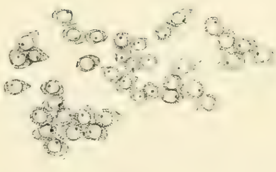
118 Cells of the corpus mammillare. Toluidin blue method. × 575.

119–121 Neurones of the corpus mammillare. Golgi method. × 93. From sagittal sections. It will be noted from figs. 117 to 121 that the cells of the corpus mammillare are very small and closely packed.

corp. mam. (*G*), corpus mammillare, the ganglion mammillare of Goldstein; *nucl. cbl. hyp.*, nucleus cerebellaris hypothalami; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. fasc. long.*, nucleus fasciculus longitudinalis medialis; *nucl. post. thal.*, nucleus posterior thalami; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *nucl. rub.* (*G*), nucleus ruber of Goldstein; *nucl. subrot.*, nucleus subrotundus; *s. mam.*, sulcus mammillaris of Goldstein; *tr. cbl. tect.*, tractus cerebello-tectalis.

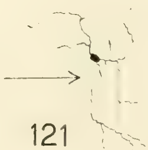
117

119



118

120



121

PLATE 33

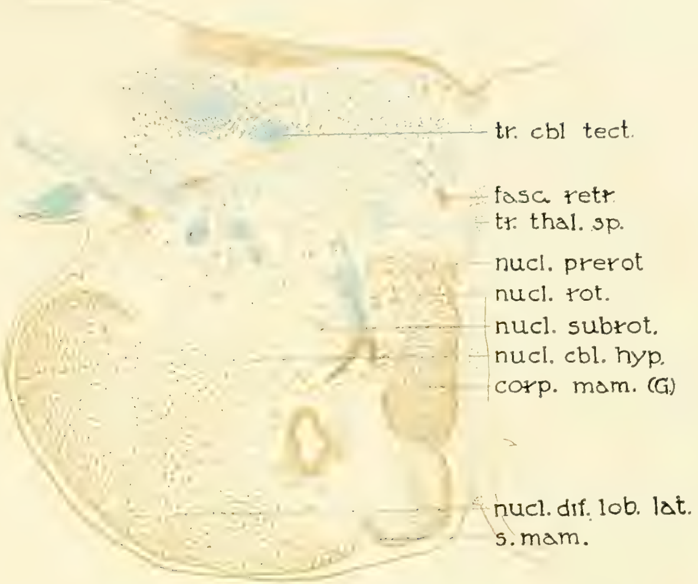
EXPLANATION OF FIGURES

122 Transection through the corpus mammillare. Weigert method. $\times$ 17.

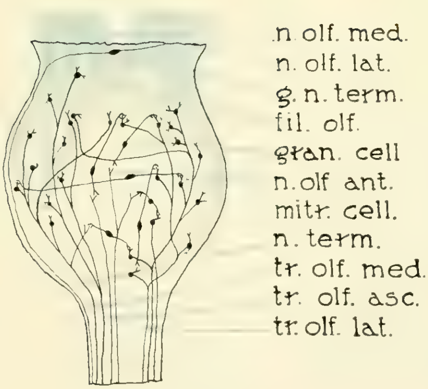
123 Diagram showing the two parts of the right olfactory nerve, and the relations of their fibers to the cells of the bulb and to the fibers of the olfactory tract.

124 Diagram of a horizontal projection of the right olfactory bulb showing the connections, in the bulb, of the different tracts of the crura.

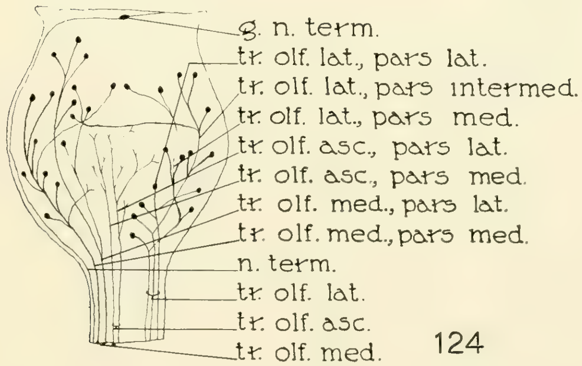
corp. mam. (*G*), corpus mammillare (of Goldstein); *fasc. retr.*, fasciculus retroflexus; *fil. olf.*, fila olfactoria; *g.n. term.*, ganglion cell of the nervus terminalis; *gran. cell*, granule cell of the nucleus olfactorius anterior; *mitr. cell,* mitral cell; *nucl. cbl. hyp.*, nucleus cerebellaris hypothalami; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *n. olf. ant.*, nucleus olfactorius anterior; *n. olf. lat.*, nervus olfactorius lateralis, consisting largely of fibers from the lamellae of the caudal and lateral portion of the olfactory capsule; *n. olf. med.*, nervus olfactorius medialis, formed largely from fibers originating chiefly in the rostral and medial portion of the capsule; *n. term.*, nervus terminalis; *nucl. prerot.*, nucleus prerotundus; *nucl. rot.*, nucleus rotundus; *nucl. subrot.*, nucleus subrotundus; *s. mam.*, sulcus mammillaris; *tr. cbl. tect.*, tractus cerebello-tectalis; *tr. olf. asc.*, tractus olfactorius ascendens, composed of centrifugal fibers from the corpus precommissurale, pars medianus; *tr. olf. asc., pars lat.*, tractus olfactorius ascendens pars lateralis; *tr. olf. asc., pars med.*, tractus olfactorius ascendens, pars medialis; *tr. olf. lat.*, tractus olfactorius lateralis, composed of the following three tracts, all centripetal, and practically all terminating, in the carp, in the nucleus olfactorius dorsalis and nucleus pyriformis of the same side; *tr. olf. lat., pars. intermed.*, tractus olfactorius lateralis, pars intermedia; *tr. olf. lat., pars lat.*, tractus olfactorius lateralis, pars lateralis; *tr. olf. lat., pars med.*, tractus olfactorius lateralis, pars medialis; *tr. olf. med.*, tractus olfactorius medialis, the mesal part of the medial olfactory radix of authors, divided into the following; *tr. olf. med., pars lat.*, tractus olfactorius medialis, pars lateralis, terminating, after decussation in the anterior commissure, in the nucleus pyriformis of the opposite side; *tr. olf. med., pars med.*, tractus olfactorius medialis, pars medialis, forming the commissura interbulbaris of most authors which, as indicated in figs. 136 and 138, is largely a decussation and not a commissure; *tr. thal. sp.*, tractus thalamo-spinalis (Kappers).


tr. cbl tect.
fasc. retr.
tr. thal. sp.
nucl. prerot
nucl. rot.
nucl. subrot.
nucl. cbl. hyp.
corp. mam. (G)
nucl. dif. lob. lat.
s. mam.

122


n. olf. med.
n. olf. lat.
g. n. term.
fil. olf.
gran. cell
n. olf ant.
mitr. cell.
n. term.
tr. olf. med.
tr. olf. asc.
tr. olf. lat.

123


g. n. term.
tr. olf. lat., pars lat.
tr. olf. lat., pars intermed.
tr. olf. lat., pars med.
tr. olf. asc., pars lat.
tr. olf. asc., pars med.
tr. olf. med., pars lat.
tr. olf. med., pars med.
n. term.
tr. olf. lat.
tr. olf. asc.
tr. olf. med.

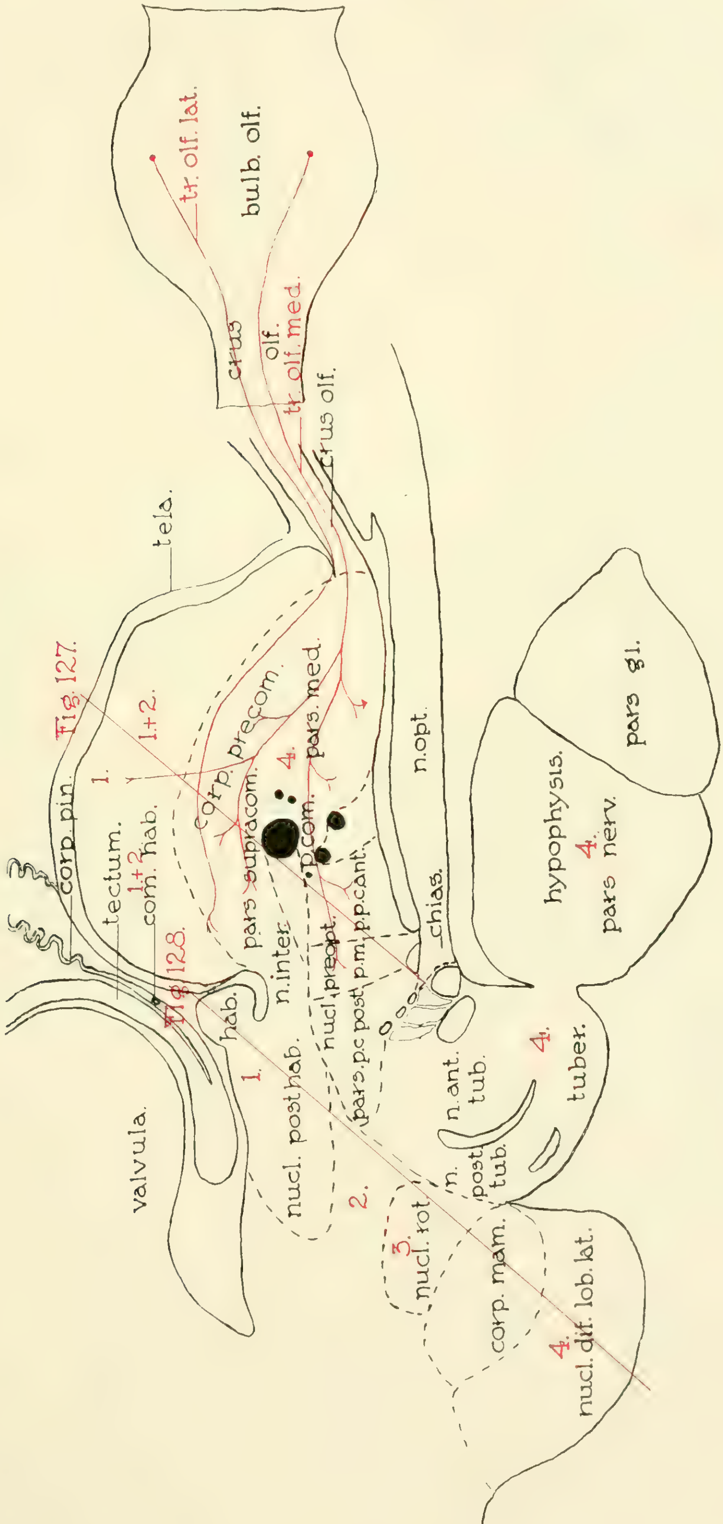
124

PLATE 34

EXPLANATION OF FIGURES

125 Diagram of a projection of the olfactory centers on a parasagittal plane, near the meson, showing the levels at which figs. 127 and 128 are taken and the relation of the different centers to the four primitive columns. × 11.

1, pars dorso-medialis hemisphaerii and epithalamus; *2*, pars dorso-lateralis hemisphaerii and pars dorsalis thalami; *3*, pars ventro-lateralis hemisphaerii and pars ventralis thalami; *4*, pars ventro-medialis hemisphaerii and hypothalamus; *tr. olf. lat.*, tractus olfactorius lateralis; *tr. olf. med.*, tractus olfactorius medialis. (For other abbreviations see explanation of fig. 141.)


bulb. olf.
tr. olf. lat.
crus olf.
tr. olf. med.
crus olf.
tela.
Fig. 127.
corp. pin.
tectum.
1+2.
com. hab.
1.
1+2.
corp. precom.
pars supracom.
4.
pars. med.
p.com.
n.opt.
chias.
n.inter.
nucl. preopt.
p.p.cant.
pars.p.c post.
p.m.
hab.
Fig. 128.
valvula.
1.
nucl. post hab.
2.
3.
nucl. rot.
n. post tub.
corp. mam.
n. ant. tub.
4.
tuber.
4.
nucl. dif. lob. lat.
hypophysis.
4.
pars nerv.
pars gl.

125

PLATE 35

EXPLANATION OF FIGURES

126 Diagram of a transection through the hemispheres of an embryonic teleost, showing the relations of the four primitive columns.

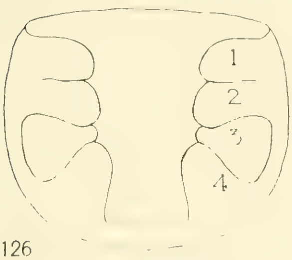
127 Diagram of a transection through the hemispheres of an adult teleost, showing the changes which have taken place through rearrangement of these columns. For approximate level see fig. 125.

128 Diagram of a transection through the diencephalon of an embryonic teleost, showing the relations of the same columns. Practically the same conditions hold in the adult. (For level see fig. 125.)

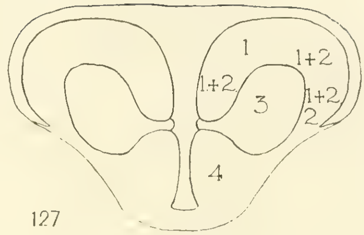
129–134 Diagrams of transections through the cerebral hemispheres of Ameiurus, at the level of the anterior commissure, showing the shifting which takes place with the gradual eversion of the tenia. Figs. 129 to 133 are camera lucida drawings obtained through the kindness of Dr. James M. Wilson, of Washington, D. C. Fig. 129, 5 mm. stage; fig. 130, 6 mm. stage; fig. 131, 9 mm. stage; fig. 132, 10 mm. stage; fig. 133, 12–13 mm. stage; fig. 134, adult.

135 Transection through the cerebral hemispheres of Amia calva in the region of the anterior commissure, to illustrate the eversion of the hemisphere wall, after Mrs. Susanna Phelps Gage ('93).

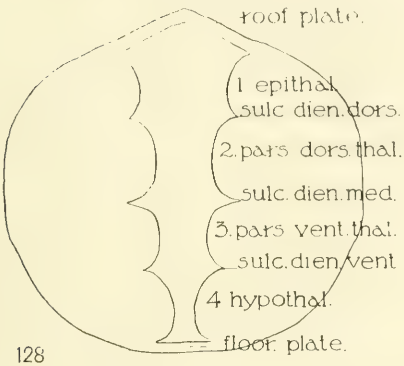
1, pars dorso-medialis hemisphaerii and epithalamus; *2*, pars dorso-lateralis hemisphaerii and pars dorsalis thalami; *3*, pars ventro-lateralis hemisphaerii and pars ventralis thalami; *4*, pars ventro-medialis hemisphaerii and hypothalamus; *epithal.*, epithalamus; *hypothal.*, hypothalamus; *pars dors. thal.*, pars dorsalis thalami; *pars vent. thal.*, pars ventralis thalami; *s. lim. tel.*, sulcus limitans telencephali; *sulc. dien. dors.* sulcus diencephalicus dorsalis; *sulc. dien. med.*, sulcus diencephalicus medialis; *sulc. dien. vent.*, sulcus diencephalicus ventralis.



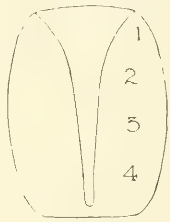
126



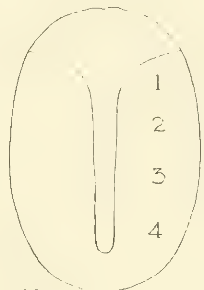
127



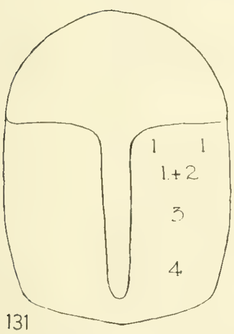
128



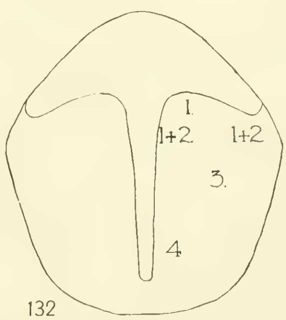
129



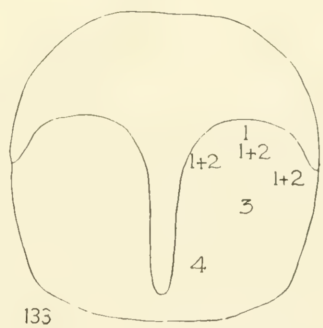
130



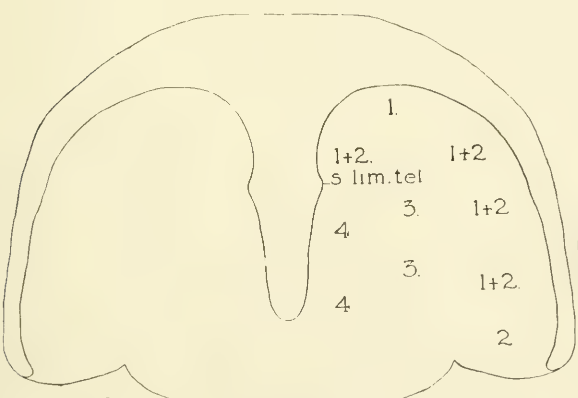
131



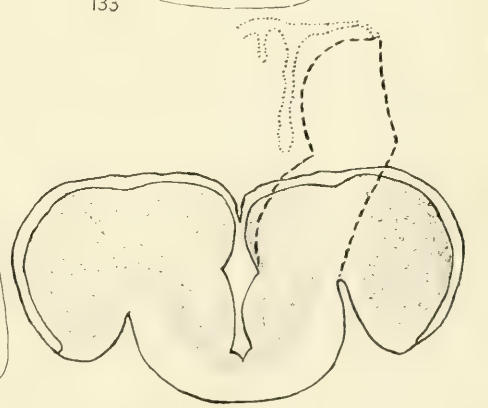
132



133



134



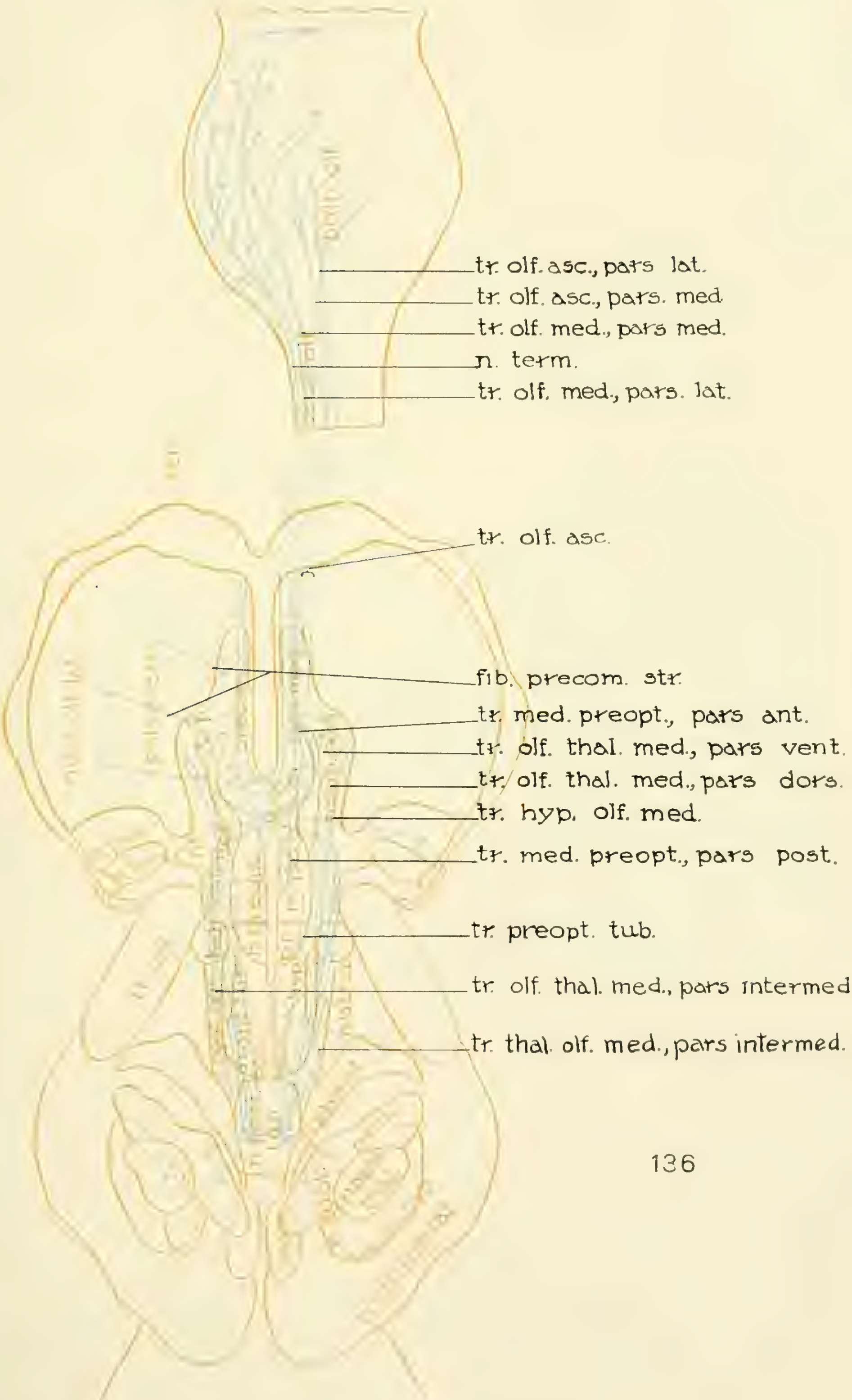
135

PLATE 36

EXPLANATION OF FIGURES

136 Diagram of a horizontal projection of the olfactory centers showing in blue the connections of the corpus precommissurale, together with the fiber components of the fasciculus medialis hemisphaerii and associated tracts. × 12.

bulb. olf., bulbus olfactorius; *c. mam.*, corpus mammillare, ganglion mammillare of Goldstein; *corp. precom.*, corpus precommissurale, consisting of the pars medianus, pars commissuralis, and pars supracommissuralis; *crus olf.*, crus olfactorium; *ent.*, nucleus entopeduncularis; *fib. precom. str.*, fibrae precommissurales striatici, running from the precommissural body to the palaeostriatum; *hab.*, ganglion habenulae; *hyp.*, hypophysis; *m.*, pars magnocellularis of the nucleus preopticus; *n. ant. tub.*, nucleus anterior tuberis; *n. cbl. hyp.*, nucleus cerebellaris hypothalami of Goldstein; *n.c.l.*, nucleus commissuralis lateralis ; *n. dif. lob. lat.*, nucleus diffusus lobi lateralis; *n. int.*, nucleus intermedius; *n. lat. tub.*, nucleus lateralis tuberis; *n. olf. lat.*, nucleus olfactorius lateralis; *n. posthab.*, nucleus posthabenularis; *n. post. thal.*, nucleus posterior thalami of Goldstein; *n. post. tub.*, nucleus posterior tuberis; *n. preopt.*, nucleus preopticus; *n. prerot.*, nucleus prerotundus; *n. pyr.*, nucleus pyriformis; *n. rot.*, nucleus rotundus; *n. subr.*, nucleus subrotundus; *n. ten.*, nucleus teniae; *n. term.*, nervus terminalis; *p.a.*, pars parvocellularis anterior of the nucleus preopticus; *paleostr.*, palaeostriatum, the striatum of most authors; *pars. com.*, pars commissuralis of the corpus precommissurale; *pars. med.*, pars medianus or the nucleus medianus of the corpus precommissurale; *p.p.*, pars parvocellularis posterior of the nucleus preopticus; *p. sup. com.*, pars supracommissuralis of the corpus precommissurale; *sac. vasc.*, saccus vasculosus; *tela*, the so-called pallium; *tr. hyp. olf. med.*, tractus hypothalamo-olfactorius medialis; *tr. med. preopt.*, *pars ant.*, tractus mediano-preopticus, pars anterior; *tr. med. preopt.*, *pars post.*, tractus mediano-preopticus, pars posterior; *tr. olf. asc.*, tractus olfactorius ascendens; *tr. olf. asc.*, *pars lat.*, tractus olfactorius ascendens. pars lateralis; *tr. olf. asc.*, *pars med.*, tractus olfactorius ascendens, pars medialis; *tr. olf.*, *med.*, *pars lat.*, tractus olfactorius medialis, pars lateralis; *tr. olf. med.*, *pars. med.*, tractus olfactorius medialis, pars medialis; *tr. olf. thal. med.*, *pars dors.*, tractus olfacto-thalamicus medialis, pars dorsalis; *tr. olf. thal. med.*, *pars intermed.*, tractus olfacto-thalamicus medialis, pars intermedia (short descending association fibers); *tr. olf. thal. med. pars vent.*, tractus olfacto-thalamicus medialis, pars ventralis; *tr. opt.*, tractus opticus; *tr. preopt. tub.*, tractus preoptico-tuberis; *tr. thal. olf. med.*, *pars intermed.*, tractus thalamo-olfactorius medialis, pars intermedia (short ascending association fibers).


tr. olf. asc., pars lat.
tr. olf. asc., pars. med.
tr. olf. med., pars med.
n. term.
tr. olf. med., pars. lat.
tr. olf. asc.
fib. precom. str.
tr. med. preopt., pars ant.
tr. olf. thal. med., pars vent.
tr. olf. thal. med., pars dors.
tr. hyp. olf. med.
tr. med. preopt., pars post.
tr. preopt. tub.
tr. olf. thal. med., pars intermed
tr. thal. olf. med., pars intermed.

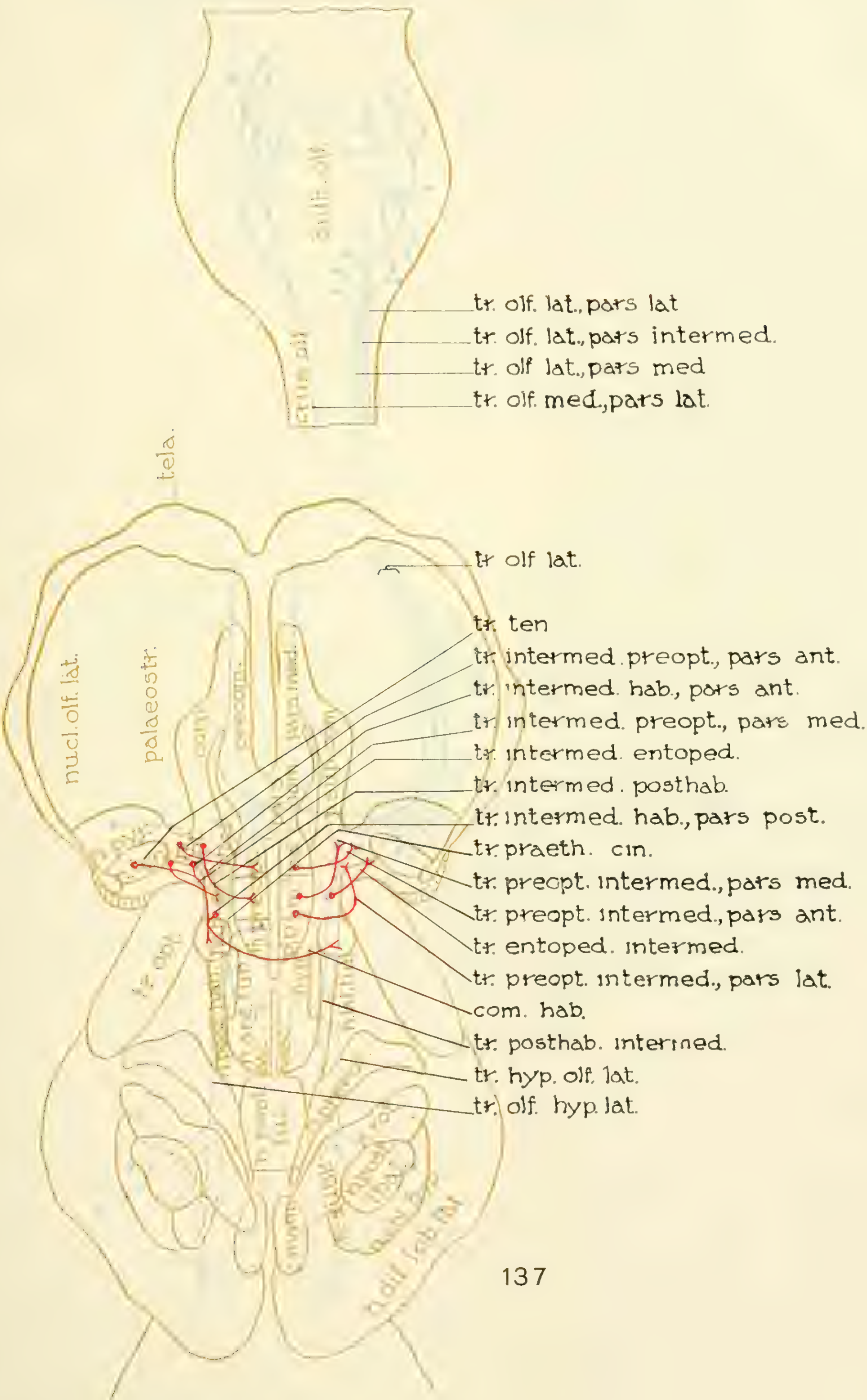
136

PLATE 37

EXPLANATION OF FIGURES

137 Diagram of a horizontal projection of the olfactory centers showing the connections of the nucleus pyriformis, nucleus teniae, nucleus olfactorius lateralis, and nucleus intermedius. On the right side are shown the tracts terminating in these nuclei, on the left side the tracts originating in them. × 12.

com. hab. (red), com. habenularum; *tr. entoped. intermed.* (red), tractus entopedunculo-intermedius; *tr. hyp. olf. lat.* (blue), tractus hypothalamo-olfactorius lateralis; *tr. intermed. entoped.* (red), tractus intermedio-entopeduncularis; *tr. intermed. hab., pars ant.* (red), tractus intermedio-habenularis, pars anterior; *tr. intermed. hab., pars post.* (red), tractus intermedio-habenularis, pars posterior; *tr. intermed. posthab.* (blue), tractus intermedio-posthabenularis; *tr. intermed. preopt. pars ant.* (red), tractus intermedio-precopticus, pars anterior; *tr. intermed. preopt., pars med.* (red), tractus intermedio-preopticus, pars medialis; *tr. olf. hyp. lat.* (blue), tractus olfacto-hypothalamicus lateralis; *tr. olf. lat.* (blue), tractus olfactorius lateralis; *tr. olf. lat., pars intermed.* (blue), tractus olfactorius lateralis, pars intermedia; *tr. olf. lat., pars med.* (blue), tractus olfactorius lateralis, pars medialis; *tr. olf. med., pars lat.* (blue), tractus olfactorius medialis, pars lateralis; *tr. posthab. intermed.* (blue), tractus posthabenulo-intermedius; *tr. praeth. cin.* (blue), tractus praethalamo-cinereus; *tr. preopt. intermed., pars ant.* (red), tractus preoptico-intermedius, pars anterior; *tr. preopt. intermed., pars lat.* (red), tractus preoptico-intermedius, pars lateralis; *tr. preopt. intermed., pars med.* (red), tractus preoptico-intermedius, pars medialis; *tr. ten.* (red), tractus teniae, the tractus olfacto-habenularis of Kappers, Goldstein, etc. (For other abbreviations see explanation of fig. 136.)


tr. olf. lat., pars lat
tr. olf. lat., pars intermed.
tr. olf lat., pars med
tr. olf. med., pars lat.
tela.
tr olf lat.
nucl. olf. lat.
palaeostr.
tr. ten
tr. intermed. preopt., pars ant.
tr. intermed. hab., pars ant.
tr. intermed. preopt., pars med.
tr. intermed. entoped.
tr. intermed. posthab.
tr. intermed. hab., pars post.
tr. praeth. cin.
tr. preopt. intermed., pars med.
tr. preopt. intermed., pars ant.
tr. entoped. intermed.
tr. preopt. intermed., pars lat.
com. hab.
tr. posthab. intermed.
tr. hyp. olf. lat.
tr. olf. hyp. lat.

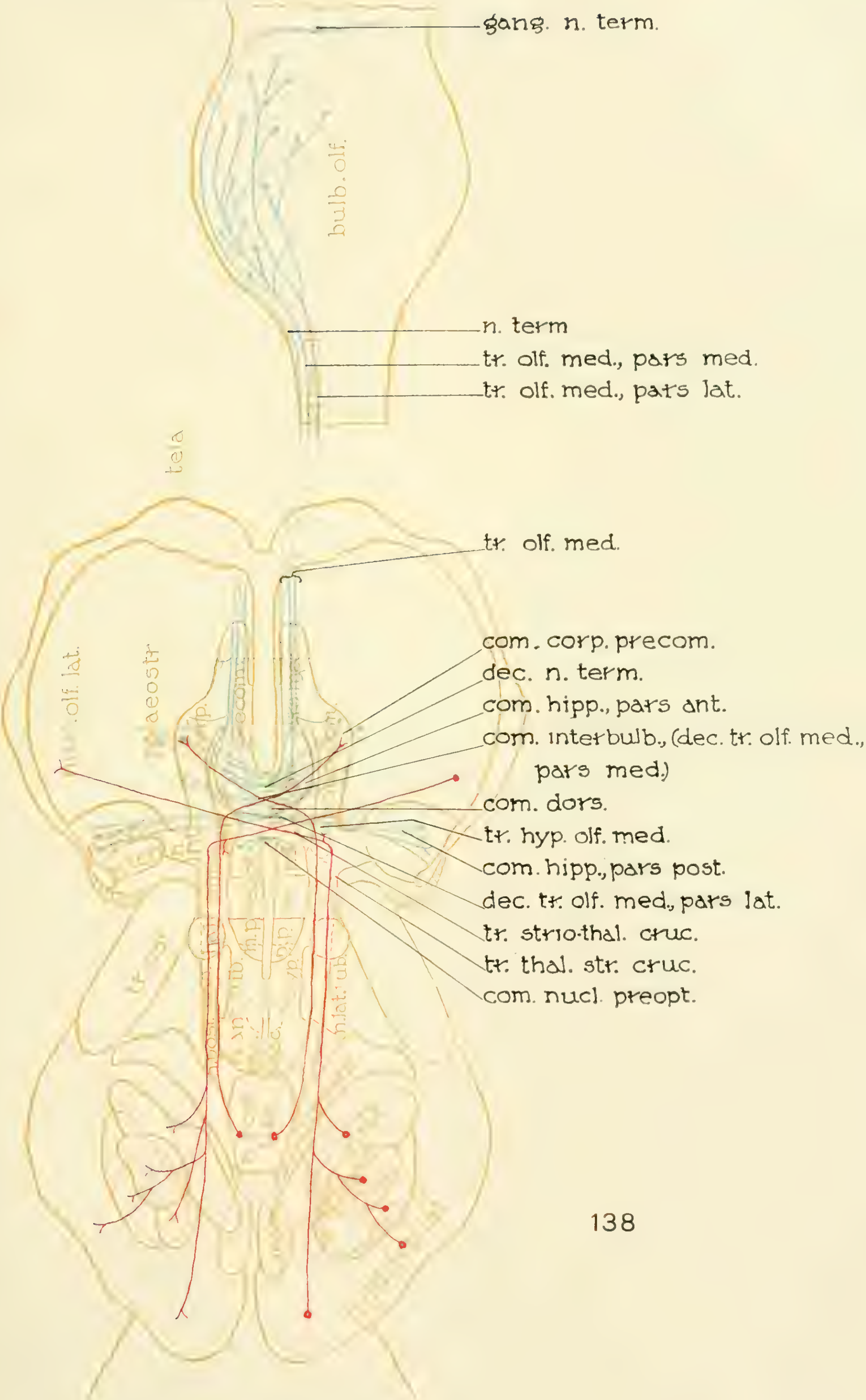
137

PLATE 38

EXPLANATION OF FIGURES

138 Diagram of a horizontal projection of the olfactory centers, showing the fibers entering into the composition of the anterior commissure, together with their connections. × 12.

com. corp. precom. (green), commissura corporium precommissuralium; *com. dors.* (green), commissura dorsalis; *com. hipp., pars ant.* (green), commissura hippocampi, pars anterior; connecting the two nuclei olfactorii dorsales or primordia hippocampi; *com. hipp., pars post.* (green), commissura hippocampi, pars posterior, the commissura internuclearis of Goldstein; *com. interbulb.*, (*dec. tr. olf. med., pars med.*) (blue), commissura interbulbaris (decussation of the tractus olfactorii mediales, partes mediales); *com. nucl. preopt.* (green), commissura nucleorum preopticorum; *dec. n. term.* (blue), decussatio nervorum terminalium; *dec. tr. olf. med., pars lat.* (blue), decussation of the tractus olfactorii mediales, partes laterales; *gang. n. term.* (blue), ganglion cell of the nervus terminalis; *n. term.* (blue), nervus terminalis; *tr. hyp. olf. med.* (red), tractus hypothalamo-olfactorius medialis; *tr. olf. med.* (blue), tractus olfactorius medialis; *tr. olf. med., pars lat.* (blue), tractus olfactorius medialis, pars lateralis; *tr. olf. med. pars med.* (blue), tractus olfactorius medialis, pars medialis; *tr. strio-thal. cruc.* (red), tractus strio-thalamicus cruciatus (shown only on one side); *tr. thal. str. cruc.* (red), tractus thalamo-striaticus cruciatus, (shown only on one side). (For other abbreviations see explanation of fig. 136.)


gang. n. term.
bulb. olf.
n. term
tr. olf. med., pars med.
tr. olf. med., pars lat.
tela
tr. olf. med.
com. corp. precom.
dec. n. term.
com. hipp., pars ant.
com. interbulb., (dec. tr. olf. med., pars med.)
com. dors.
tr. hyp. olf. med.
com. hipp., pars post.
dec. tr. olf. med., pars lat.
tr. strio-thal. cruc.
tr. thal. str. cruc.
com. nucl. preopt.

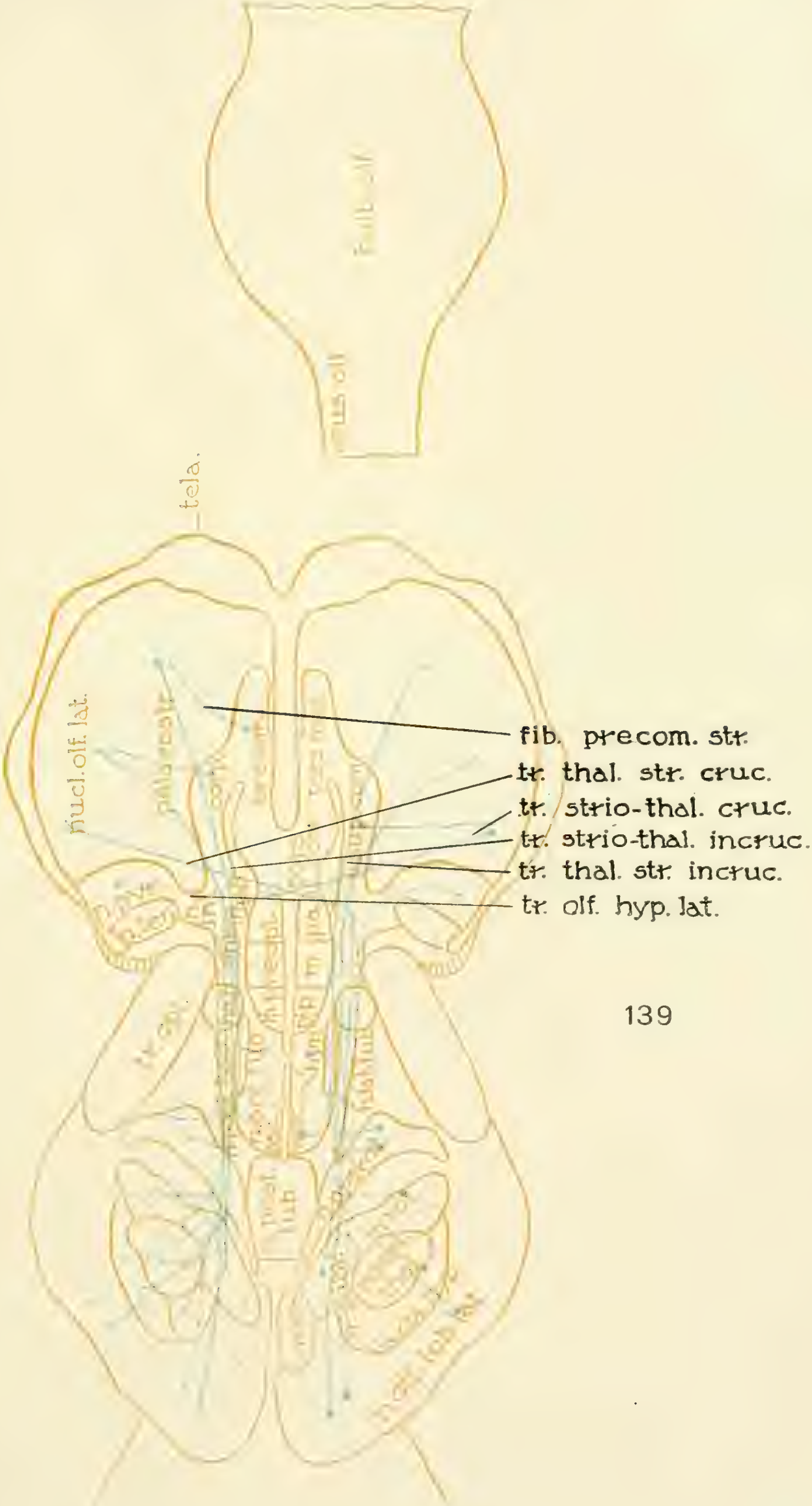
138

PLATE 39

EXPLANATION OF FIGURES

139 Diagram of a horizontal projection of the olfactory centers showing in blue the pathways of the fibers entering into the composition of the fasciculus lateralis hemisphaerii. The ascending fibers are shown on the right, the descending on the left. × 12.

fib. precom. str., fibrae precommissurales striatici; *tr. olf. hyp. lat.*, tractus olfacto-hypothalamicus lateralis; *tr. strio-thal. cruc.*, tractus strio-thalamicus cruciatus; *tr. strio-thal. incruc.*, tractus strio-thalamicus incruciatus; *tr. thal. str. cruc.*, tractus thalamo-striaticus cruciatus; *tr. thal. str. incruc.*, tractus-thalamo-striaticus incruciatus. (For other abbreviations see explanation of fig. 136.)


fib. precom. str.
tr. thal. str. cruc.
tr. strio-thal. cruc.
tr. strio-thal. incruc.
tr. thal. str. incruc.
tr. olf. hyp. lat.
tela
nucl. olf. lat.

139

PLATE 40

EXPLANATION OF FIGURES

140 Diagram of a horizontal projection of the olfactory centers showing the connections of the postcommissural olfactory nuclei, with the exception of the habenular fibers shown on fig. 142. Fibers which terminate in these centers are shown on the right, while those which originate from them appear on the left. × 12.

com. nucl. preopt. (red), commissura nucleorum preopticorum; *tr. entoped. preopt.* (red), tractus entopedunculo-preopticus; *tr. intermed. preopt., pars ant.* (red), tractus intermedio-preopticus, pars anterior; *tr. intermed. preopt., pars med.* (red), tractus intermedio-preopticus, pars medialis; *tr. lat. preopt.* (red), tractus lateralis preopticus; *tr. med. preopt., pars ant.* (blue), tractus mediano-preopticus, pars anterior; *tr. med. preopt., pars post.* (blue), tractus mediano-preopticus, pars posterior; *tr. olf. med., pars lat.* (blue), tractus olfactorius medialis, pars lateralis (see fig. 136); *tr. posthab. preopt.* (red), tractus posthabenulo-preopticus; *tr. praeth. cin.* (blue), tractus praethalamo-cinereus; *tr. preopt. entoped.* (red), tractus preoptico-entopeduncularis; *tr. preopt. intermed., pars ant.* (red), tractus preoptico-intermedius, pars anterior; *tr. preopt. intermed., pars lat.* (red), tractus preoptico-intermedius, pars lateralis; *tr. preopt. intermed., pars med.* (red), tractus preoptico-intermedius, pars medialis; *tr. preopt. lat.* (red), tractus preoptico-lateralis; *tr. preopt. posthab. pars ant.* (red) tractus preoptico-posthabenularis, pars anterior; *tr. preopt. posthab., pars post.* (red), tractus preoptico-posthabenularis, pars posterior; *tr. preopt. tub.* (blue), tractus preoptico-tuberis. (For other abbreviations see explanation of fig. 136.)

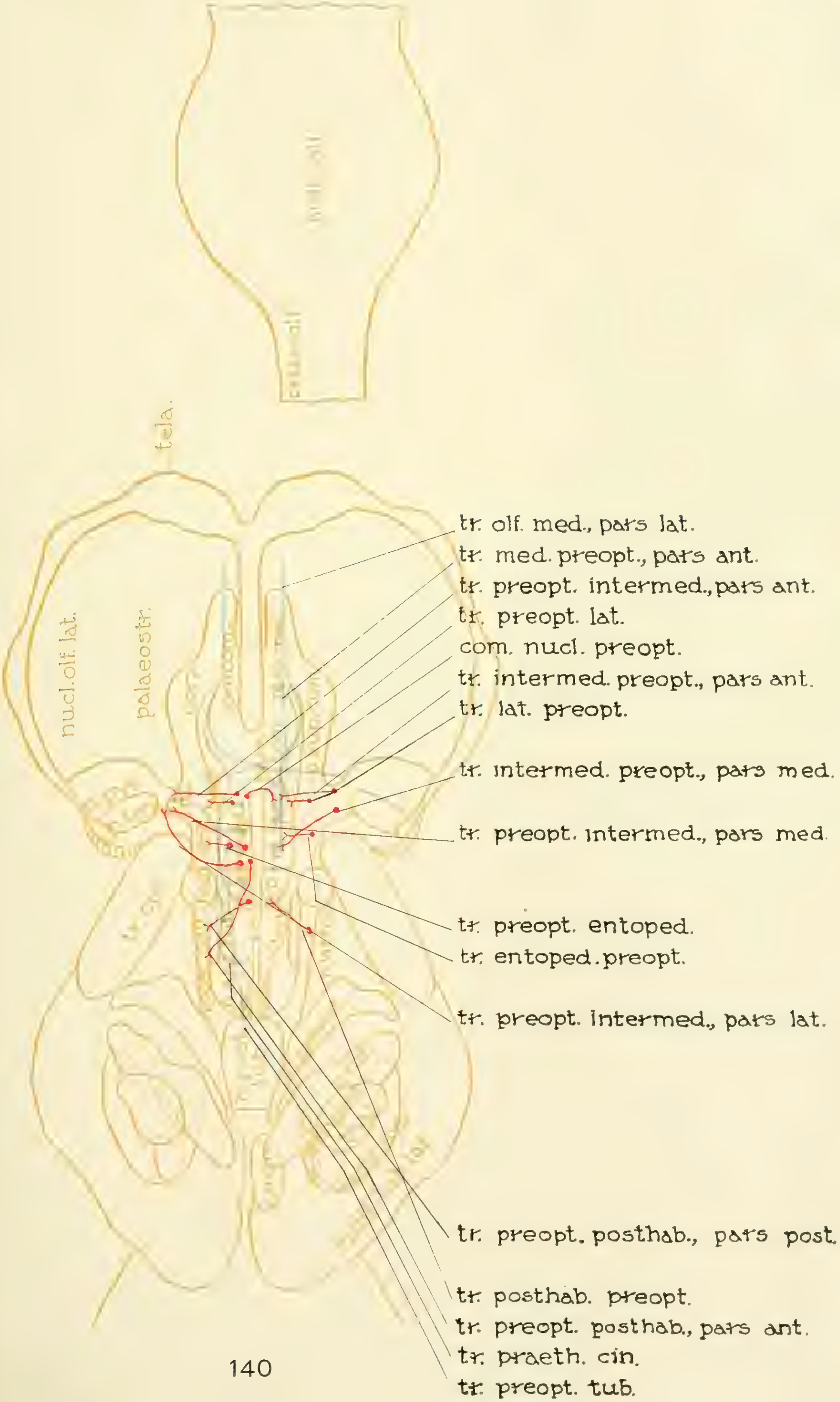

tela.
nucl. olf. lat.
palaeostr.
tr. olf. med., pars lat.
tr. med. preopt., pars ant.
tr. preopt. intermed., pars ant.
tr. preopt. lat.
com. nucl. preopt.
tr. intermed. preopt., pars ant.
tr. lat. preopt.
tr. intermed. preopt., pars med.
tr. preopt. intermed., pars med.
tr. preopt. entoped.
tr. entoped. preopt.
tr. preopt. intermed., pars lat.
tr. preopt. posthab., pars post.
tr. posthab. preopt.
tr. preopt. posthab., pars ant.
tr. praeth. cin.
tr. preopt. tub.

PLATE 41

EXPLANATION OF FIGURES

141 Diagram of a projection of the olfactory centers on a para-sagittal plane near the meson, showing the components of the tractus olfacto-habenularis, and their connections, in black (*cf. Goldstein, Taf. 11, fig. 7*). × 12.

chias., optic chiasma; *com. corp. precom.* + *com. hipp. pars ant.*, commissura corporium precommissuralium plus commissura hippocampi, pars anterior; *com. hab.*, commissura habenularum; *com. Herrick*, commissura Herricki; *com. horiz.*, commissura horizontalis; *com. interbulb.* (*dec. tr. olf. med., pars med.*) commissura interbulbaris (decussation of the tractus olfactorii mediales, partes mediales); *com. nucl. preopt.*, commissura nucleorum preopticorum; *com. trans.*, commissura transversa; *corp. mam.*, corpus mammillare; *corp. pin.*, corpus pineale; *corp. precom.*, corpus precommissurale; *crus olf.*, crus olfactorium; *dec. n. term.*, decussatio nervorum terminalium; *dec. tr. hyp. olf. med.* + *dec. tr. olf. med., pars lat.* + *com. dors.* + *com. hipp., pars post.*, decussation of the tractus hypothalamo-olfactorii mediales, plus decussation of the tractus olfactorii mediales, partes laterales, plus commissura dorsalis, plus commissura hippocampi, pars posterior; *fasc. retr.*, fasciculus retroflexus; *fib. ans.*, fibrae ansulatae; *hab.*, ganglion habenulae; *n. ant. tub.*, nucleus anterior tuberis; *n. opt.*, nervus opticus; *n. post. tub.*, nucleus posterior tuberis; *nucl. dif. lob. lat.*, nucleus diffusus lobi lateralis; *nucl. posthab.*, nucleus posthabenularis; *nucl. preopt.*, nucleus preopticus; *nucl. rot.*, nucleus rotundus; *pars gland.*, pars glandularis of the hypophysis; *pars med.*, pars medialis of the corpus precommissurale; *pars nerv.*, pars nervosa of the hypophysis; *pars p.c. post.*, pars parvocellularis posterior of the nucleus preopticus; *p.m.*, pars magnocellularis of the nucleus preopticus; *p. p. c. ant.*, pars parvocellularis anterior of the nucleus preopticus; *p. supracom.*, pars supracommissuralis of the corpus precommissurale; *tectum*, tectum mesencephali; *tela*, so-called pallium; *tr. dien. hab.*, tractus diencephalo-habenularis; *tr. entoped. hab.*, tractus entopedunculo-habenularis; *tr. hab. dien.*, tractus habenulo-diencephalicus; *tr. intermed. hab., pars ant.*, tractus intermedio-habenularis, pars anterior; *tr. intermed. hab., pars post.*, tractus intermedio-habenularis, pars posterior; *tr. olf. hab.*, tractus olfacto-habenularis; *tr. posthab. hab.*, tractus posthabenulo-habenularis; *tr. preopt. hab., pars ant.*, tractus preoptico-habenularis, pars anterior; *tr. preopt. hab., pars lat.*, tractus preoptico-habenularis, pars lateralis; *tr. preopt hab., pars med.*, tractus preoptico-habenularis, pars medialis; *tr. preopt. hab., pars post.*, tractus preoptico-habenularis, pars posterior; *tr. strio-thal. cruc.*, decussation of the tractus striothalamici cruciati; *tr. teniae*, tractus teniae; *valvula*, valvula cerebelli. (See also fig. 125.)

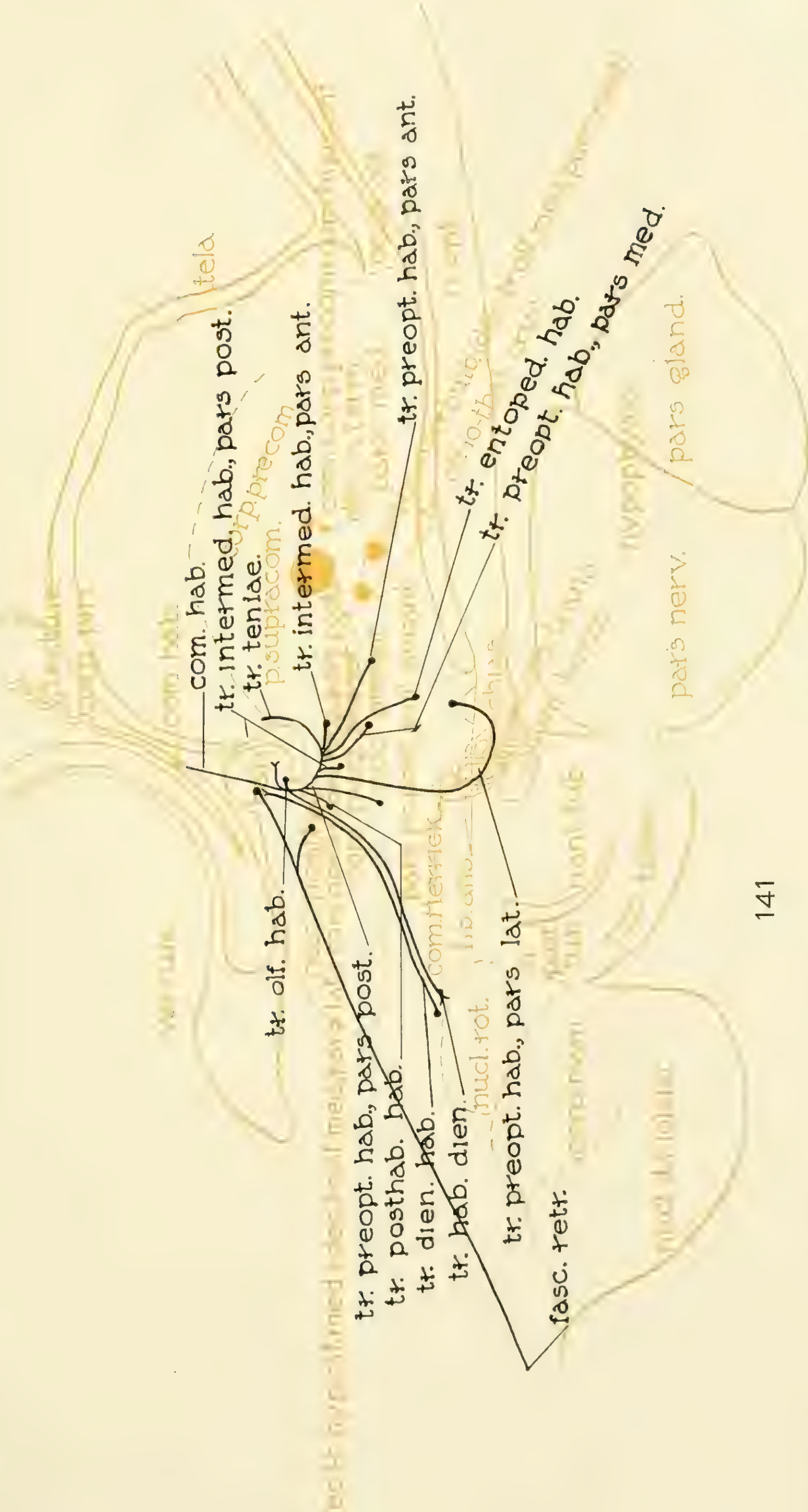
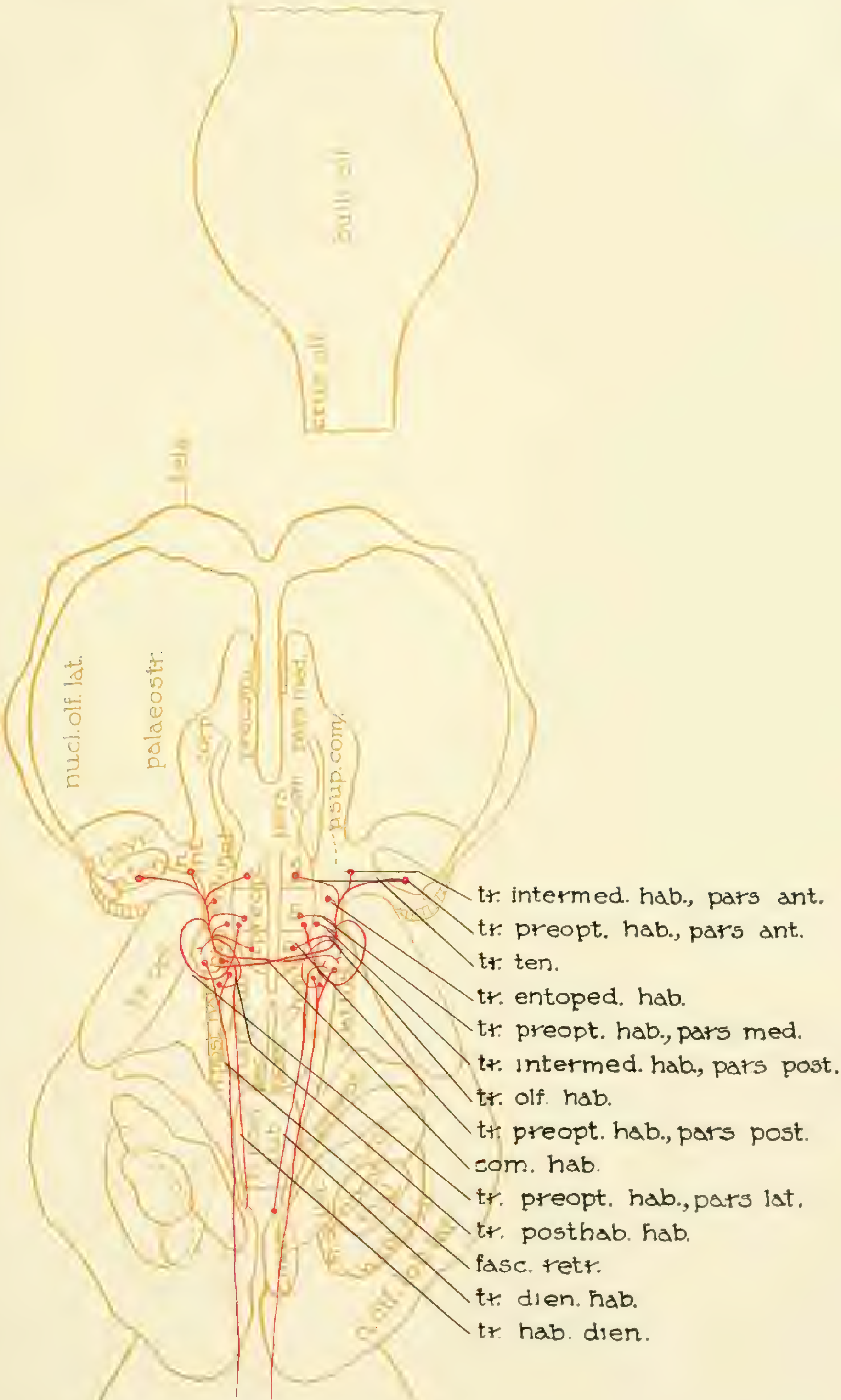

com. hab.
tr. intermed. hab., pars post.
tr. teniae.
tr. intermed. hab., pars ant.
tr. preopt. hab., pars ant.
tr. entoped. hab.
tr. preopt. hab., pars med.
tela
p. supracom.
pars gland.
pars nerv.
tr. olf. hab.
tr. preopt. hab., pars post.
tr. posthab. hab.
tr. dien. hab.
tr. hab. dien.
tr. preopt. hab., pars lat.
com. Herrick
nucl. rot.
fasc. retr.
141

PLATE 42

EXPLANATION OF FIGURES

142 Diagram of a horizontal projection of the olfactory centers showing, in red, the components of the tractus olfacto-habenularis and their connections. $\times$ 12.

com. hab., commissura habenularum, or commissura superior; *fasc. retr.*, fasciculus retroflexus, Meynert's bundle, tractus habenulo-peduncularis; *tr. dien. hab.*, tractus diencephalo-habenularis (a portion of the tractus habenulo-diencephalicus of Goldstein); *tr. entoped. hab.*, tractus entopedunculo-habenularis; *tr. hab. dien.*, tractus habenulo-diencephalicus; *tr. intermed. hab., pars. ant.*, tractus intermedio-habenularis, pars anterior; *tr. intermed. hab., pars post.*, tractus intermedio-habenularis, pars posterior; *tr. olf. hab.*, tractus olfacto-habenularis (under this name are included all the fiber systems which ascend into the habenula of either side); *tr. posthab. hab.*, tractus posthabenulo-habenularis; *tr. preopt. hab., pars ant.*, tractus preoptico-habenularis, pars anterior; *tr. preopt. hab., pars lat.*, tractus preoptico-habenularis, pars lateralis; *tr. preopt. hab., pars med.*, tractus preoptico-habenularis, par medialis; *tr. preopt. hab., pars post.*, tractus preoptico-habenularis, pars posterior; *tr. ten.*, tractus teniae (the tractus olfacto-habenularis of Kappers, Goldstein, etc.). (For other abbreviations see explanation of fig. 136.)


nucl. olf. lat.
palaeostr.
p.sup.com.
tr. intermed. hab., pars ant.
tr. preopt. hab., pars ant.
tr. ten.
tr. entoped. hab.
tr. preopt. hab., pars med.
tr. intermed. hab., pars post.
tr. olf. hab.
tr. preopt. hab., pars post.
com. hab.
tr. preopt. hab., pars lat.
tr. posthab. hab.
fasc. retr.
tr. dien. hab.
tr. hab. dien.

142

THE TELENCEPHALON IN CYCLOSTOMES[1]

J. B. JOHNSTON

From The Institute of Anatomy, University of Minnesota

FORTY-ONE FIGURES

The telencephalon of cyclostomes presents in many ways more primitive conditions than are known in other vertebrates. Cyclostomes are therfore important in the effort to trace the phylogeny of various structures in the cerebral hemispheres. The discussions regarding the interpretation of the lateral lobes, the pallium and the ventricles (Ahlborn, Rabl-Rückhard, Studnicka, Edinger) together with the contributions by Sterzi ('09) and the writer ('02a), have made clear the significance of most parts of the telencephalon at least in Petromyzonts. The lateral lobes are true hemispheres,[2] containing lateral ventricles connected with the third ventricle by wide interventricular foramina. The hemispheres are pushed back against the sides of the diencephalon by pressure from the buccal apparatus. The bulbar formation occupies the broad rostral wall of the hemisphere and does not project forward as a bulbus olfactorius. In addition to this bulbar formation the hemisphere walls include secondary olfactory centers and a basal-central area heretofore known as corpus striatum. The bulbar formation and secondary olfactory centers are separated by a groove which represents the olfactory peduncle.

The anterior portion of the third ventricle is closed above and rostrally by a membrane which is thickened in two places by commissures (figs. 5. 6). Rostral to the extraordinarily thick

[1] Neurological Studies from the Institute of Anatomy, University of Minnesota, No. 16.

[2] The writer here uses the term hemisphere as synonymous with the lateral lobe or evagination of the telencephalon, accepting in this the suggestion of Professor Herrick ('10, p. 492).

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chiasma ridge the lamina terminalis extends from the preoptic recess to the recessus neuroporicus, rostral to the interventricular foramen. The position of the recessus neuroporicus was first clearly established by Sterzi ('07). The lamina terminalis is thickened by the anterior commissure. Above the recessus neuroporicus the roof of the ventricle is thickened by the so-called dorsal olfactory commissure or decussation. This thickened lamina should be called the lamina supraneuroporica (Burckhardt '94 b, '94 c, '07, Johnston, '11 b). A short distance caudal to this lamina occurs a small fold in the membranous roof which was shown by Sterzi ('07) to be the velum transversum. Caudal to this a long dorsal sac extends to the superior commissure and habenular bodies. This dorsal sac is covered dorsally by the parapineal body and epiphysis, which depress the sac in various degrees.

Upon the general morphology of the petromyzont telencephalon thus far there is general agreement among workers. There are, however, disputed questions regarding the relations of the telencephalon and diencephalon, and the location of the primordium of the cortical area of higher vertebrates.

The writer has reviewed the preparations previously studied and has examined new preparations of the same and other species of petromyzonts. The writer wishes to express his sincere thanks to Professor Gage for a very generous supply of ammocoetes and adults of both Lampetra and Petromyzon dorsatus. Other specimens of Lampetra have been obtained from Professor Reighard's laboratory both while the writer was located there and later through the kind assistance of Dr. L. J. Cole. The writer is deeply indebted to Professor Charles Brookover for the loan of the series of sections of Ichthyomyzon which is described beyond. Thanks are also due to Professor Reighard for the loan of a series of sections of a new dwarf lamprey not yet described. Through the kindness of Mr. W. F. Allen of this laboratory I have had the opportunity to section and study several stages of the ammocoetes of the Pacific coast lamprey, Entosphenus.

The di-telencephalic boundary

The writer has attempted ('09) an accurate definition of the boundary in question, upon the basis of selachian, amphibian, avian and mammalian embryos. The result was to show that the optic chiasma belongs to the telencephalon, the boundary being defined by the velum transversum above and the caudal surface of the chiasma ridge below. It was shown that the telencephalon includes, in addition to the hemispheres, a median portion surrounding the rostral part of the third ventricle. The floor of this telencephalon medium is occupied by the optic chiasma. Its roof (roof plate of His) is made up of the lamina terminalis, lamina supraneuroporica and tela chorioidea. The telencephalon constitutes a complete brain ring or segment as His contended, although shorter than His thought. The hemispheres are lateral evaginations of this telencephalon medium. In the series of vertebrates, as in the stages of the ontogeny, a progressively larger part of the telencephalon is evaginated into the hemispheres, until in man only the chiasma ridge and the small region between it and the lamina terminalis remains as the telencephalon medium.

This definition of the di-telencephalic boundary has been accepted by Herrick ('10), Kappers and Carpenter ('11), and others, and its substantial correctness will be assumed for the purposes of this paper. If the di-telencephalic boundary is to be determined in cyclostomes in the same way as in other vertebrates, it becomes necessary only to describe the velum transversum in cyclostomes accurately and completely, since the chiasma ridge is already well understood.

The recognition by Sterzi of the small fold behind the dorsal decussation as the homologue of the velum transversum of higher forms was a valuable contribution to the interpretation of the cyclostome brain. The velum was recognized, however, only in median sections and the postition of the velum in the median plane does not define the boundary between the telencephalon and diencephalon. The writer has shown at length elsewhere ('11 a, '11 b) the errors and inconsistencies which arise from taking into account only the position of the velum in the median plane. It

is the point of attachment of the velum to the massive walls which determines the boundary between telencephalon and diencephalon. The velum transversum is a fold of the tela chorioidea having the form of an arch whose pillars rest on the massive lateral walls. This point of attachment is the meeting place of the taenia thalami and taenia fornicis. The position of the velar arch in the median plane depends upon the form of the tela chorioidea of the third ventricle as affected by the general form of the brain and the pressure or traction exerted upon the tela by surrounding structures. Thus in the selachian brain the velum is nearly transverse and vertical in position (fig. 1) and is attached to the lateral walls just in front of the habenular bodies. In certain ganoids, on the other hand, the velum is a very deep fold which is inclined forward at an angle greater than 45 degrees. The pillars of the velum are attached to the lateral walls just rostral to the habenular bodies exactly as in selachians (fig. 2). The point of attachment of the velum to the lateral walls may be more sharply defined with reference to the internal structure of the massive wall. It is just at the point of attachment of the velum that the several categories of fibers which make up the stria medullaris converge into a compact bundle to ascend to the habenular nucleus and commissure (commissura superior Osborn). In amphibians and reptiles this portion of the lateral wall, to which the velum is attached and which is traversed by the stria medullaris, Herrick ('10, p. 419) has called the eminentia thalami. This low eminence is clearly seen in figures 1 and 2, but is not lettered.

A careful study of the velum transversum in Lampetra shows that it is attached as in fishes and amphibians immediately rostral to the habenular bodies and that its point of attachment is the dorsal border of the eminentia thalami.

As is well known, the right habenular body is very much larger than the left, and each is bounded ventrally by a deep groove, the sub-habenular sulcus (figs 3, 5, 6). At its rostral end this deep sulcus leads into the dorsal sac. Here it meets with a vertical sulcus which descends in the brain wall and curves forward to enter the interventricular foramen (figs. 5, 6, sulcus limitans hippocampi). The common space formed by the union of these two

deep sulci where they join in the caudal and lateral angle of the dorsal sac is a very deep and narrow cleft which may be called the recessus praehabenularis (figs. 14, 18). This deep recess has been formed probably by the crowding together of the brain due to pressure from in front. Below the habenular body two nearly vertical ridges are seen (figs. 5, 6) in the side wall of the thalamus. The more caudal one is occupied by the tractus habenulo-peduncularis (figs. 21, 22). It is much more prominent on the right, owing to the greater size of the nucleus habenulae and the fiber tract on the right side. The more cephalic ridge is about equally developed on the two sides and contains the stria medullaris (figs. 20, 21). This I shall call the eminentia thalami. It is bounded in front by the sulcus limitans hippocampi and extends up in the recessus praehabenularis as a narrow ridge (figs. 20, 26, 27). The groove separating the two ridges corresponds to the sulcus *b* of the selachian brain (Johnston '11 a) and to the sulcus diencephalicus medius of Herrick ('10) in amphibians.

The dorsal ridge in front of the habenular body is large in Lampetra and presents a slight but distinct eversion similar to that in the teleost brain (figs. 7, 30, 31). To this ridge the writer formerly ('02 a) gave the name epistriatum. In anticipation of the results of the following pages, we may call it here the primordium hippocampi. It extends forward over the interventricular foramen where it becomes continuous with the roof of the lateral evagination.

In order to determine whether this ridge belongs to the telencephalon or diencephalon we must discover the point of attachment of the velum transversum to the massive walls. This point is difficult to determine in most petromyzonts because the velum is very rudimentary and is recognizable only near the median line. This is true of the adults of Petromyzon dorsatus and Lampetra which the writer has examined, of Ichthyomyzon studied by Herrick and apparently of the forms studied by European authors. In the ammocoetes of Petromyzon dorsatus, however, the velum is better developed and appears as a fold which extends across the whole width of the tela so that the attachment to the massive walls can be determined.

In order to demonstrate the pillars of the velar arch in this form the writer has made a plate reconstruction of the dorsal part of the telencephalon and diencephalon. Owing to the compression of the dorsal sac by the epiphysis and parapineal body the velum shows to best advantage in sagittal sections and the reconstruction was made from these (fig. 8). The model was made from the left side of the brain and included the recessus neuroporicus in front and a part of the nucleus habenulae behind. In order to see as much of the velum as possible the model has been drawn from a medio-ventro-caudal direction. This figure should be compared with figure 3, which shows the left half of the forebrain from a model of another specimen of Petromyzon dorsatus (ammocoetes). The cut surface along the upper border of the figure corresponds very nearly to the median sagittal plane. In this plane is seen the fold described by Sterzi as the velum transversum. Extending caudo-laterally from this above the primordium hippocampi is a fold of the tela which continues without interruption into the extreme caudo-lateral angle of the dorsal portion of the ventricle. The deep cleft in which the velum is seen in the figure is the recessus praehabenularis described above. In the depth of this recess the lateral pillar of the velar arch is attached to a small ridge which is identified as the eminentia thalami by the presence in it of the stria medullaris. As the model is viewed from an unusual angle, the relations will perhaps be more clear by comparison with figures 23 to 27, which represent five sections of the series from which the model was constructed. The relations of the recessus praehabenularis and of the eminentia thalami in Lampetra are shown in figures 14 to 22. Here the sulcus praehabenularis is narrower than in the ammocoetes because of the enlargement and crowding of the surrounding parts. The disposition of the velum transversum in Petromyzon dorsatus is essentially the same as that in ganoids and teleosts. The velum is not only inclined far forward as in the latter fishes, but owing to the depression of the dorsal sac by the overlying epiphysis, its middle part is pressed down far below the plane of attachment of its pillars. The whole course of the velum is diagrammatically represented in figure 28 as it might be seen in the dorsal

aspect of the brain. As indicated in this figure, that portion of the dorsal ventricular space which lies above the velum transversum is properly called the dorsal sac, while the portion below the velum belongs to the telencephalic portion of the third ventricle.

This description of the velum transversum makes it possible to define clearly the boundary between the telencephalon and diencephalon. It is marked, as shown in figures 5 and 6, by a line running from the attachment of the velum transversum upon the dorso-rostral border of the eminentia thalami to the caudal surface of the chiasma ridge.

The ventricular sulci in diencephalon and telencephalon

Attention has been called above to the sulcus limitans hippocampi, the dorsal or sub-habenular sulcus and the sulcus *b* or sulcus medius. In figure 5 are to be seen three other important ventricular grooves, the sulcus limitans of His, the sulcus hypothalamicus and a sulcus connecting the recessus praeopticus with the foramen interventriculare. The presence of the last named in embryos and adults of other classes of vertebrates has been pointed out ('11 a). The further study of slightly evaginated brains (cyclostomes, ganoids and teleosts) makes it necessary to withdraw the view stated earlier (in '11 a, p. 45) that the sulcus arising in the preoptic recess is the continuation of the sulcus limitans hippocampi.

The sulcus hypothalamicus is seen a short distance behind the interventricular foramen diverging ventrally from the sulcus limitans hippocampi. It grows deeper and descends into the hypothalamus as a crescentic groove. Discussion of the question whether this sulcus or any part of it is comparable to the sulcus hypothalamicus of the human brain is reserved. The name is used here in a purely descriptive sense and is evidently appropriate. The sulcus is the same as Herrick's sulcus diencephalicus ventralis. This name has not been adopted because the sulcus in all lower vertebrates is a transverse rather than a longitudinal sulcus.

The sulcus limitans of His traverses the midbrain in the same position as in other vertebrates and meets the sulcus hypothalamicus over and in front of the tuberculum posterius. It can not be

traced forward to the preoptic recess, apparently owing to the great prominence of the chiasma-ridge and the supra-optic nucleus.

Professor Herrick has given an account of the sulci in Ichthyomyzon which differs from the above account in important respects. With Professor Herrick's kind permission I reproduce his figure 73 as figure 29 of this paper. Comparison of this with my figure 5 representing the model of the Lampetra forebrain shows that Herrick has given the name sulcus diencephalicus medius to a part of my sulcus hypothalamicus and that he has described the lower end of this sulcus hypothalamicus as a separate sulcus under the name of the sulcus diencephalicus ventralis. He recognizes also a sulcus subhabenularis and sulcus diencephalicus dorsalis.

As it was impossible to harmonize this description with the condition in Lampetra, I have secured for study at Professoı Herrick's suggestion the identical series of sections from which his drawings were made. The sections are fifteen microns thick and with the exception of a single broken section the series is perfect. A model has been made at a magnification of 100 diameters, the right half of which is drawn from the ventricular surface in figure 6. The model when finished was a trifle longer than it should be, in the proportion of 94 to 90.

Referring to Herrick's figures 74 to 81, it should be noted that Professor Herrick viewed the sections from in front, so that the right side of the brain appears in the left side of his figures. Thus the right nucleus habenulae, which is the larger, appears on the left side in figures 80 and 81. From this it follows that the reconstruction in figure 73 (figure 29 of this paper) represents the ventricular surface of the right half of the brain as if it were the left half. This is mentioned only to show that the model figured is properly to be compared directly with Herrick's reconstruction.

The model shows the following points. The primordium hippocampi is not so large as in Lampetra but has the same form and relations. The nucleus habenulae projects rostıad somewhat over the primordium hippocampi. The sulcus limitans hippocampi and sulcus hypothalamicus very closely resemble those in Lampetra. The eminentia thalami is better marked than in Lampetra, being a prominent ridge near the nucleus habenulae.

Behind the eminentia thalami is a ridge extending from the nucleus habenulae to the interpeduncular region and occupied by the tractus habenulo-peduncularis. Between this ridge and the eminentia thalami is a sulcus diencephalicus medius which is somewhat more regular than in Lampetra. The sulcus limitans of His is somewhat less pronounced than in Lampetra.

Professor Herrick thought that he found in Ichthyomyzon conditions which supported his theory regarding the division of the diencephalon and the telencephalon of amphibians into four longitudinal columns. In amphibians (Herrick, '10, p. 419) the sulcus medius forms the dorsal boundary of the eminentia thalami and runs caudally toward the tuberculum posterius. The statement that this sulcus and the sulcus ventralis 'converge anteriorly to the interventricular foramen' is evidently without foundation, since the sulcus medius is situated dorso-caudal to the eminentia thalami and the velum transversum, and can not reach the interventricular foramen. In the amphibians studied by the writer (Amblystoma, Necturus, Cryptobranchus, Rana, Bufo) the sulcus medius runs up into the dorsal sac and has no relation to the interventricular foramen. This fact is clearly shown also in Herrick's figures 5, 18, 19, 22, 33 and 34.

In Herrick's Ichthyomyzon figures 78, 79 and 80, the *s.m.* corresponds to my sulcus limitans hippocampi, while in figure 81 and on the right side of figure 80, *s.m.* is the sulcus hypothalamicus. Consistent with his indentification of this with the sulcus medius, Herrick labels the area below it as the pars ventralis thalami and compares it with the eminentia thalami of amphibians ('10, p. 471). This interpretation is obviously untenable, since the eminentia thalami of amphibians is caudo-dorsal to the interventricular foramen, immediately adjacent to the nucleus habenulae, is bounded below by the sulcus diencephalicus ventralis and is traversed by the compact stria medullaris just before this bundle enters the nucleus habenulae. See Herrick's figures 17–to 22. The true position of the eminentia thalami in Ichthyomyzon is clear from Herrick's figures 80 and 81 in which the compact stria medullaris is about to enter the nucleus habenulae. These figures show that the eminentia thalami lies as in amphibians at

the dorsal border of the brain near the nucleus habenulae. This is more clear from my figure 9 which is drawn from a section between the two drawn in Herrick's figures 80 and 81. In this section the stria medullaris is seen in the eminentia thalami. In Herrick's figure 81 the stria medullaris has bent laterad into the outer portion of the nucleus habenulae and the tractus habenulo-peduncularis is coming down near the ventricle. Having thus identified the eminentia thalami, it is evident that the groove in Ichthyomyzon which corresponds to the sulcus medius of amphibians is the groove so lettered in figure 6.

Professor Herrick has completely overloooked the greater part of the sulcus limitans hippocampi in Ichthyomyzon, probably because the greater part of its course lies more or less parallel with the plane of the transverse sections. For the same reason he failed to recognize that his *s.m.* and *s.v.* were the two ends of one and the same crescentic groove. The model clearly shows the true relations in both cases, and readily explains how natural Professor Herrick's interpretation was in the absence of models.

In dealing with the relations of the telencephalon and diencephalon in the dorsal region, Professor Herrick ('10, pp. 473–4) says,

> On account of the very small degree of evagination of the cerebral hemisphere in cyclostomes the di-telencephalic fissure is shallow and the pars dorsalis thalami passes over without interruption into the lateral wall (lobus olfactorius) of the hemisphere. Moreover this fissure does not extend upward to the mid-dorsal line and thus the dorso-median ridge is able to pass continuously from one segment to the other. In higher vertebrates this fissure extends dorsally up to the site of the velum transversum and it is so deep as to interrupt the continuity of both the ridge and all other massive tissue of the pars dorsalis thalami with their telencephalic representatives.

It is necessary to define clearly what is meant by the di-telencephalic fissure. In the human and mammalian brain there is a great groove or fissure between the posterior part of the hemisphere and the thalamus, midbrain and cerebellum. Near the bottom of this is the chorioidal fissure of the hemisphere. Dorsally the fissures of the two sides join in the great longitudinal fissure. Taken together these constitute in a true sense a margi-

ginal or limiting fissure of the hemispheres. It owes its existence to the fact that the evagination of the hemisphere causes an angle or fold between its wall and the wall of the brain stem. The whole fissure may therefore be referred to under the descriptive term, stem-hemisphere fissure. When lower vertebrates are examined it is seen that the stem-hemisphere fissure is well-marked in reptiles and amphibians, but in true fishes is only a broad shallow groove or constriction. In Petromyzonts, one of the most striking features is the sharp separation between hemisphere and brain stem (fig. 7). In a previous paper ('09) the writer has shown that this is not the line of division between telencephalon and diencephalon. The groove seen in figure 7 running from near the median line in front outward and backward is the stem-hemisphere fissure. It marks the boundary between the hemisphere and the telencephalon medium, not only in cyclostomes but in all classes of vertebrates. When the hemispheres expand and lie apposed to each other in the median plane, this forms the great longitudinal fissure and its lateral extension between the posterior pole of the hemisphere and the brain stem.

Obviously this stem-hemisphere fissure can not be called a di-telencephalic fissure. The writer has suggested ('09, p. 516) that the di-telencephalic fissure owes its origin to the withdrawal of tissue to form the optic vesicle. In this Professor Herrick concurs ('10, p. 467). The di-telencephalic fissure lies at the junction of the telencephalon medium and diencephalon, while the hemisphere evagination takes place some distance farther rostrad, and the two are entirely independent. That this is so is perfectly clear from cyclostomes, selachians and other fishes. The evagination of the hemispheres has, therefore, nothing to do with the di-telencephalic fissure or the continuity of diencephalon and telencephalon. Further, the di-telencephalic fissure is dorsal in position from the start and does not extend farther dorsally in higher vertebrates. What does happen is that in higher vertebrates more and more of the telencephalon medium comes to be evaginated into the hemispheres until the stem-hemisphere fissure gradually approaches the di-telencephalic fissure.

In cyclostomes the di-telencephalic fissure is as clearly present as in other vertebrates. It is marked by the eminentia thalami to which the velum transversum is attached. In the ammocoetes of Petromyzon dorsatus, at least, the di-telencephalic boundary is further marked by an external groove (fig. 4). Professor Herrick is in error in his speculations regarding the continuity of the dorsal part of the thalamus and the telencephalon in cyclostomes (pp. 474 and 477–8). The di-telencephalic fissure is slightly masked in cyclostomes because of the crowding back of the telencephalon against the diencephalon but the sulcus medius comes to the dorsal border here precisely as in amphibians and if the dorsal column is interrupted by the di-telencephalic fissure in amphibians, it is interrupted in just the same way in cyclostomes.

Herrick states (p. 472) that he has indicated in figure 73 by a dotted line (*s.d.*) 'a somewhat arbitrary boundary' between his dorso-median ridge (my primordium hippocampi) and his pars dorsalis thalami. This dotted line does not correspond to the sulcus limitans hippocampi or to any thing that I can find in Lampetra, Petromyzon dorsatus, Entosphenus or Ichthyomyzon. The dotted line is lettered sulcus diencephalicus dorsalis while in the text (p. 470) the dorsal is spoken of as synonymous with the sub-habenular sulcus. In amphibians (Herrick '10, 431 and fig. 22) the subhabenular and dorsal sulci are distinct but are regarded as two parts of a sulcus which separates the epithalamus from the dorsal part of the thalamus. In amphibians the dorsal is the more caudal segment of the common sulcus. In the Ichthyomyzon diagram the positions are reversed. In amphibians neither of these sulci has even a remote relation with the interventricular foramen, and both lie wholly within the diencephalon. In the Ichthyomyzon diagram the line *s.d.* is connected with the foramen and lies wholly within the telencephalon. This arbitrary line in Ichthyomyzon has therefore no relation to the sulcus diencephalicus dorsalis of amphibians.

Herrick regards the groove which extends rostrad from the foramen as a continuation of the sulcus diencephalicus dorsalis. In this position there are two grooves in Ichthyomyzon and in Lampetra. One extends forward from the dorsal angle of the

foramen, the other from the ventral angle, and the two converge into the neuroporic recess. The lower one of these grooves is the one designated by Herrick as the telencephalic extension of the sulcus dorsalis (Herrick '10, figs. 75, 76). The dorso-median ridge (my promordium hippocampi) ends rostrally in the sections which contain the so-called dorsal olfactory commissure. As seen in the model, it is very abruptly reduced in dorso-ventral thickness at the foramen and extends over the foramen only as a slender strand of cells (which appears in Herrick's fig. 77) in close connection with the commissure. Rostral to the foramen this slender ridge disappears entirely and the larger ridge which Herrick calls the dorso-median ridge in his figures 75 and 76 contains glomeruli and olfactory fibers and belongs to the formatio bulbaris. Of the two sulci extending rostrally from the foramen, the upper one separates the dorso-median ridge (primordium hippocampi) from the formatio bulbaris, the lower one separates the formatio bulbaris from the medial olfactory nucleus.

In the ammocoetes of Petromyzon dorsatus (figs. 3, 8) there is only one groove extending from the foramen to the neuroporic recess. This sulcus separates the primordium hippocampi from the medial olfactory nucleus and there is no formatio bulbaris in this position. Lampetra presents an intermediate condition. The two grooves are nearer together and the area of formatio bulbaris which abuts on the ventricle is less than in Ichythyomyzon. These facts show that in Ichthyomyzon and Lampetra the evagination of the hemisphere has not completely carried out the formatio bulbaris, but that a part of this formation remains in the telencephalon medium and forms part of the wall of the median ventricle rostral to the foramen. In Petromyzon dorsatus the evagination of the formatio bulbaris is complete. Consequently, the two sulci in Ichthyomyzon and Lampetra may be regarded as merged into one sulcus in Petromyzon dorsatus. This one sulcus begins in the neuroporic recess and passes into the rostral wall of the lateral ventricle, separating the primordium hippocampi from the medial olfactory nucleus. The position of this sulcus is the same as that of the medial zona limitans (hippocampi) in selachians (Johnston, '11 a).

The brain of Professor Reighard's dwarf lamprey presents larger hemispheres with wider lateral ventricles than I have seen in any other petromyzont. In the form and size of the primordium hippocampi and in the disposition of the ventricular sulci it agrees well with Lampetra.

The primordium hippocampi

The description of the ventricular sulci has made clear the definite ventral boundary of this body on the ventricular side. Externally a very deep groove separates it from the caudal pole of the hemisphere (fig. 7). At the interventricular foramen the primordium hippocampi bends through the roof of the foramen to become directly continuous with the roof of the hemisphere. Caudally, the sulcus limitans separates this body sharply from the eminentia thalami and nucleus habenulae internally, but on the external surface there is no visible boundary in adult Lampetra. In the ammocoetes of Petromyzon dorsatus the external surface shows a vertical groove which marks the caudal boundary of the telencephalon (fig. 4).

The neurones of this body belong to a special type which is found nowhere else in the brain of Lampetra. The same type of neurone is characteristic of the primordium hippocampi of ganoids and amphibians. As far as the writer's studies have gone, these neurones are as truly characteristic of the primordium hippocampi as are the Purkinje cells of the cerebellum, and are much more highly differentiated in cyclostomes than are the Purkinje cells.

In a fomer paper ('02 a, p. 40) these neurones as they appear in Golgi preparations were discribed as follows:

> The cells of the epistriatum are arranged in two to four rows adjoining the cavity. The larger end of the pyramidal cell body is next the cavity and a large dendrite which arises from the apex divides into two or more large branches which expand in the fiber layer. The dendrites bear numerous small spines which are knobbed at the end (fig. 26) in the manner characteristic of the epistriatum, inferior lobes, and tectum of Acipenser. These peculiar spines are found nowhere in the brain of Petromyzon except on the epistriatum cells. These cells are so closely similar to the epistriatum cells of Acipenser that it would be impossible to mistake their identity.

The pyramidal body and the dendrites studded with knobbed spines are so characteristic of these neurones that the resemblance to the hippocampal cells in Acipenser, Amia and Rana is at once striking and unequivocal. Examples of these cells in each of the forms named are shown in figures 30 to 34, for comparison with the cells in Lampetra. The bodies of the neurones in Lampetra stand near the ventricle and their dendrites divide into a few relatively straight branches which traverse the thickness of the wall, often reaching the outer surface (figs. 30, 31).

When we look for the boundary between the primordium hippocampi and the epithalamus, the internal structure as seen in horizontal sections seems to furnish the necessary data. First, there is no difference in the internal structure of the whole dorso-median ridge bounded by the foramen and the sulcus limitans hippocampi. Everywhere it is filled by the peculiar type of neurones just described and nowhere is there any change of finer structure which would lead us to say that any two or more parts of it represent different functional centers. However, the moment the sulcus limitans hippocampi is passed in any direction we come upon neurones of types wholly different from those of this ridge. This can not be accidental; it must be the expression of functional differentiation.

The neurones of the so-called striatum have been described and figured in my earlier paper ('02 a, figs 18, 19). They are bipolar or multipolar cells with irregularly curved and branching dendrites free from spines. The neruones of the nucleus habenulae have also been figured ('02 a, fig. 16; this paper, figs. 17 to 20). They are, like those in Acipenser, small cells with short very irregular dendrites often with enlarged tips bearing tufts of small branches. The subhabenular region is broadly continuous with the primordium hippocampi but the boundary line is very distinct in Golgi sections. The type of neurones peculiar to the primordium hippocampi stops abruptly along a line drawn latero-caudad from the sulcus limitans hippocampi (see figures of horizontal sections, 18, 19, 20). At the same time along this same line end abruptly the parallel fibers coursing lengthwise through this ridge. At this line the fibers are cut off in horizontal sections because they are

turning up into the nucleus habenulae, as is seen in the most dorsal sections. Compare figures 15, 16 and 19, 20. Behind the line mentioned there is a wholly irregular tangle of nerve fibers and farther back the fibers of the optic tract. Scattered among the tangled fibers are bipolar and multipolar neurones with long sinuous dendrites devoid of spines. The type of structure of the two bodies as a whole is as strikingly different as are the individual neurones found in them.

In transverse sections, owing to the direct contiguity and the oblique overlapping of the primordium hippocampi and the epithalamus, the boundary is of course not so clear, but there is a very abrupt transition from one type of neurones to the other which strongly suggests the distinctness of the two centers.

Tretjakoff ('09) gives a very imperfect description of the 'praethalamus' without figures. He states that its cells are much like those of the thalamus and that the only afferent or efferent tract connected with the praethalamus is constituted by the fibers from the parapineal organ. These reach the praethalamus after crossing in the habenular commissure. The praethalamus serves as a rudimentary perception center for the parapineal eye. "Eine andere Bedeutung des Präthalamus lässt sich bei Ammocoetes kaum vermuten, da ungeachtet der grossen Zahl der Zellen der Präthalamus von Ammocoetes, nach meinen Untersuchungen, keine eigenen aus- oder zu führenden Bahnen hat." The author was evidently impressed with the inadequacy of the parapineal tract to account for so large a center with a great number of cells. This impresses us much more when we remember that the parapineal organ and tract exist only on the left side. According to Tretjakoff's description the fibers cross in the commissure to enter the (right) praethalamus. Hence the left 'praethalamus' is wholly devoid of afferent fibers according to this author, and we are led to suppose that a large center, rich in highly developed cells exists in Ammocoetes totally without function. The writer has found no evidence in his preparations that the parapineal tract enters the 'praethalamus'.

Schilling ('07) is inclined to the opinion that the 'praethalamus' belongs to the telencephalon (p. 431; Herrick cites him erroneously

on p. 473) and points out that it is separated from the nucleus habenulae by a deep ventricular sulcus. He did not distinguish the characteristic cells of the 'praethalamus,' but describes the passage through it of the bundles of the taenia thalami which give off collaterals to it.

None of the authors who have studied the petromyzont brain have used methods adequate to the differentiation of the types of cells characteristic of the so-called praethalamus and the parts adjacent to it. No method is so well adapted to this purpose as the Golgi method and the description of this region given by the writer in 1902 stands as the most complete heretofore given. That description gave, however, a very incomplete account of the facts shown in my preparations and the deficiency is to some extent made up in the figures accompanying this paper. The 'praethalamus' of authors is sharply distinguished from the epithalamus and thalamus not only by the ventricular sulci but by the well marked characteristics of its cells and by the course of fibers which traverse it.

Between this body and the roof of the hemisphere there is in the same way a clear difference in the type of cells. This has been sufficiently illustrated earlier ('02 a) and there is no dispute among authors upon this point.

The fiber tracts related to the primordium hippocampi

These fiber tracts have been very imperfectly understood. Both Schilling ('07, p. 432) and Tretjakoff ('09, p. 731) state that no tracts connected with this nucleus were seen.

The stria medullaris is perhaps less complex in petromyzonts than in the higher fishes, but at least four bundles related to the telencephalon are present. (Fibers connecting the epithalamus with other parts of the diencephalon will not be considered here). One of these bundles comes from the medial olfactory nucleus, enters the primordium hippocampi near its rostral end above the foramen and seems to pass over the dorsal ventricular surface of this body to enter the nucleus habenulae (figs. 14 to 19, 25, 26, 35). It is by no means certain that this is a continuous tract.

A tract from the medial olfactory nucleus over the foramen interventriculare to the nucleus habenulae is not known in other vertebrates. The fibers passing up from the medial olfactory nucleus as far as the primordium hippocampi occupy the same place as the tractus olfacto-corticalis septi in selachians and amphibians. If these fibers end in the primordium hippocampi, then what appears to be the continuation of them to the nucleus habenulae must be classed as cortico-habenular fibers. A second bundle comes from the lateral and caudal walls of the hemisphere, passes up behind the foramen through the inner and lower part of the primordium hippocampi to join with the first as it enters the nucleus habenulae (figs. 17, 18, 19). This is the tractus olfacto-habenularis of authors. A third bundle comes from the preoptic region and joins the first two (fig. 35). These three bundles unite into a large compact bundle which traverses the eminentia thalami at the bottom of the prehabenular recess and enters the nucleus habenulae (figs. 19, 26, 27). The fourth component of the stria medullaris is very diffuse and comes from the whole thickness of the primordium hippocampi. Axones arising from the cells of the primordium are seen in many cases passing at first peripherally among the dendrites of these cells and then turning to run toward the nucleus habenulae through the substance of the primordium hippocampi. It is these fibers in addition to the first and second bundles above described that give a longitudinal striation to the whole of this body. At the caudal end of the primordium these fibers enter the nucleus habenulae caudal and somewhat dorsal to the compact bundle of the stria medullaris (figs. 16, 17).

The left nucleus habenulae is very small in Lampetra and nearly all the left stria medullaris enters the superior commissure. In the preparations from which figures 14 to 22 were drawn no fibers were seen ending in the left nucleus. The view expressed in the writer's paper on Acipenser ('01, p. 115) that the larger size of the right nucleus is correlated with the ending of a larger portion of the tractus olfacto-habenularis in the right nucleus is strongly supported in Lampetra. The superior commissure presents two main divisions, a more rostral, compact bundle and a more caudal portion made up of several strands (figs. 17, 18).

The rostral bundle is composed of fibers from the first bundle described above and its fibers seem to end in the fiber-mesh of the right nucleus (figs. 18, 19). The corresponding bundle of the right side meets with the left and seems to end with it in the right nucleus.

The strands of the more caudal division of the commissure are made up of fibers of the remaining three bundles intermingled. Many of these fibers end in the fiber mesh of the right nucleus but many others pass through the nucleus without ending. This is especially evident in such horizontal sections as those drawn in figures 17 to 20. At least a part of these fibers clearly appear to be those which arise in the primordium hippocampi. If so, it is probable that these fibers end in the corresponding body on the other side and are homologous with the posterior pallial commissure which is prominent in selachins, ganoids, teleosts and amphibians.

The fibers in the caudal division of the commissure which end in the right nucleus may include those parts of the tractus olfacto-habenularis which in other fishes arise in the lateral olfactory nucleus and in the nucleus praeopticus. These may also include fibers from the primordium hippocampi which would belong to the tractus cortico-habenularis.

It seems reasonably certain from the study of these Golgi sections that the stria medullaris contains the equivalent of the tractus olfacto-habenularis lateralis and posterior, and the commissura palli posterior as described in the selachian brain. Nearly all of these fibers from the left side cross in the superior commissure.

The writer has described ('02 a, pp. 38, 40) two afferent tracts ending in the primordium hippocampi; one ascending from the hypothalamus and one coming from the formatio bulbaris. The former has since been called tractus pallii in all fishes and is regarded as the ascending gustatory tract entering the telencephalon for the sake of correlation of gustatory with olfactory impulses. This tract decussates in the post-optic commissure and ascends over the internal face of the tractus opticus to enter the primordium hippocampi from below and behind.

The fibers which enter the rostral end of the primordium hippocampi from the formatio bulbaris are of course part of the olfactory tract. In the previous description ('02a, p. 40) it was stated that these come chiefly from the opposite side, crossing in the olfactory decussation. The contribution of the commissure to the fiber bundle entering the primordium hippocampi is illustrated in figure 24. Further study, both of the preparations used at that time and of new preparations, has shown, however, that a much larger number of direct fibers are present than was previously thought. These fibers are soon mingled with the fibers of the first and second bundles of the stria medullaris mentioned above and can not be distinguished with certainty. It is clear, however, that many fibers which enter from in front break up into end branches in the primordium hippocampi. Since we know of no other animals in which olfactory tract fibers run without relay to the nucleus habenulae, the presumption is strong that these olfactory tract fibers end in the primordium hippocampi. This is the more probable in view of the fact that in selachians corresponding fibers are present which end in the primordium hippocampi in part on the same side and in part after crossing in a commissure situated above the neuroporic recess.

Schilling and Tretjakoff both state that the fibers of the tractus olfacto-habenularis give collaterals to the 'praethalamus.' Such endings would represent a rudimentary tractus olfacto-corticalis. On comparative grounds the presumption is strong that those fibers mentioned above which run up form the medial olfactory nucleus rostral to the interventricular foramen to enter the primordium hippocampi must constitute a tractus olfacto-corticalis.

The efferent tract from the primordium hippocampi has thus far been very imperfectly understood. The writer described fibers descending to the 'striatum' and these have been confirmed by Tretjakoff. These fibers are fine and do not appear in sufficient numbers in my preparations to warrant the conclusion that they represent the chief or only efferent path of this highly differentiated nucleus. Neither is it clear that they end in the 'striatum'. It is possible that they pass through the 'striatum' but are not impregnated beyond. Other fibers may descend in the tractus pallii, as this tract in selachians and ganoids contains descending

fibers. The pathway by which the fornix columns run in all higher classes is occupied by a broad bundle of fibers connecting the medial olfactory nucleus with the primordium hippocampi (see above). If any fibers descend through this to reach the hypothalamus, they would represent the fornix columns. We must await further investigations upon these points.

We may now summarize the evidence for the interpretation of this 'praethalamus' or 'dorso-median ridge,' which has been assumed in using the name primordium hippocampi.

(a) Its caudal end is just in front of the eminentia thalami to which the velum transversum is attached. It is therefore wholly within the telencephalon.

(b) It contains highly developed and specialized cells of a type which is characteristic of the primordium hippocampi in selachians ganoids, teleosts and amphibians.

(c) It is bounded by a ventricular sulcus which agrees closely in position with the sulcus limitans hippocampi of selachians, ganoids and teleosts.

(d) Along the line of this sulcus there is a sudden change from the characteristic cells to cells of very different form in the thalamus, epithalamus and hemisphere. This abrupt change of structure is comparable to the zona limitans of fishes and amphibians.

(e) It is traversed by a part of the tractus olfacto-habenularis as in ganoids and teleosts.

(f) It has true commissural fibers passing through the superior commissure as in fishes and amphibians(commissura pallii posterior).

(g) It receives from in front fibers of the olfactory tract, direct and crossed, comparable to those in selachians and in part to those of ganoids and amphibians.

(h) It receives a tractus pallii ascending from the hypothalamus as in all fishes. The center is therefore to be regarded as an olfacto-gustatory correlation center.

(i) It appears probable that there is a tertiary olfactory tract ending in this body (tractus olfacto-corticalis).

Admitting such uncertainty as exists in the present state of our knowledge regarding the posterior pallial commissure and the tertiary olfactory connections, we have here a body of evidence

which leaves no reasonable ground for doubt that the body in question is the homologue of the primordium hippocampi as described in fishes and amphibians.

There is no ground whatever for Professor Herrick's assumption ('10, p. 473) that the greater part of this body belongs to the epithalamus, while a smaller rostral portion represents the primordium hippocampi.

The fact of greatest interest regarding this body is, perhaps, that the primordium of the visceral cortical complex of higher animals (hippocampus, fornix, and related structures) remains in cyclostomes in the telencephalon medium. As has been pointed out in previous papers ('10 c, '11 a) the hemisphere evagination in vertebrates involves at first only the primary olfactory centers and it is only in later stages of phylogeny that the cortical substance is evaginated.

Anterior pallial commissure

This has been known as the dorsal olfactory decussation, the dorsal part of the anterior commissure, and by other names. Schilling speaks of this as the anterior commissure and makes only minor mention of the true anterior commissure. Schilling recognizes in this dorsal commissure fibers connecting the formatio bulbaris of the two sides and fibers connecting each formatio bulbaris with the opposite lobus olfactorius. The writer has described fibers connecting the lobus of one side with the opposite primordium hippocampi. In addition, there are to be mentioned fibers which come to the commissure from the area of union of the caudal wall of the hemisphere with the telencephalon medium (fig. 22). A certain determination of the nature of these fibers depends on the further study of the region from which they arise. They may correspond to the fibers in selachians to which the writer has given the name corpus callosum. The matter of greatest moment in this connection is the existence in the lowest order of vertebrates of a commissure located in the lamina supraneuroporica. The writer has shown elsewhere that the corresponding commissure in selachians is large and important, and must reiterate the view that this dorsal or supraneuroporic commissure is primitive and

fundamental in vertebrates. A further examination of the pallial commissures in reptiles and mammals with reference to this is in progress (see '10 c).

Anterior commissure

This commissure is rather small in cyclostomes. It lies in the lamina terminalis in front of the preoptic recess as in all vertebrates. Its constitution is not at all clearly known. Its fibers probably come from the basal (lateral) olfactory area and the so-called 'striatum.'

The 'striatum'

The region to which the name striatum has been applied includes the supraoptic portion of the telencephalon medium and an adjacent part of the hemisphere (figs. 5, 6, 22). It is clear that this corresponds roughly to the striatum of other fishes. The writer has shown ('02 a, p. 41) that the descending fibers from the 'striatum' go to the thalamus and not to the hypothalamus. From this region fibers pass through the anterior pallial commissure, as noted above. These facts suggest that the striatal region corresponds to or contains the equivalent of the somatic area of selachians. Any decision on this point must await further study.

Mode of evagination of hemispheres. Zona limitans

The cyclostomes taken in comparison with higher forms give clear evidence of the principle announced by the writer ('10 c, '11 a) that the hemispheres are lateral evaginations of the telencephalon which have gradually involved the formatio bulbaris, the lobus olfactorius and the pallium in the order named. The comparison of the cyclostomes with selachians and amphibians with reference to the mode by which the more fully evaginated brains have come to have their present form, is very important for the interpretation of these higher brains.

If attention be given to the models shown in figures 5, 6 and 7, it will be seen that the further evagination of the hemisphere involves the turning out of the primordium hippocampi and the

striatal area through the foramen into the caudal portion of the hemisphere. Somewhat more than the rostral half of the hemisphere is occupied by formatio bulbaris. The further evagination would enlarge the caudal part of the hemisphere until it became larger than the rostral part. At the same time the hemisphere is drawn forward by the elongation of the rostrum, the olfactory bulb becomes separated form the caudal part of the hemisphere and a longer or shorter tractus olfactorius or olfactory peduncle is established.

The relations of the primordium hippocampi to the roof of the enlarging hemisphere are especially interesting. We have pointed out that there is an abrupt change of structure between the primordium and the roof of the hemisphere which may be called a zona limitans. As the primordium hippocampi pushes out into the roof of the hemisphere this zona limitans would be placed successively farther and farther laterad in this roof. In selachians ('11 a) this zona limitans runs from the olfactory peduncle backward along the dorso-lateral wall of the lateral ventricle. For a long time in the phylogeny there remains a portion of the primordium hippocampi in the telencephalon medium extending along the dorsal border of this region to the point of attachement of the velum transversum to the eminentia thalami. In this position, where the whole of the primordium is located in cyclostomes, there exists in Chimaera ('10 d) and selachians ('11 a) a slender ridge of gray matter accompanied by the fibers of the posterior pallial commissure. In ganoids and teleosts ('11 b), where the evagination of the hemispheres has been arrested, the primordium has retained in the caudal part of the telencephalon essentially the relation which it holds in cyclostomes and has taken part in the eversion of the forebrain. In amphibians the evagination has gone farther than in selachians, the primordium hippocampi occupying the roof portion of the hemisphere and the zona limitans being carried farther down in the lateral wall. There is still left in the telencephalon medium, however, a small part of the primordium hippocampi corresponding to that in selachians, although shorter (Johnston, '06). Herrick ('10, p. 476) has endeavored to show that this structure belongs to the eminentia thalami. It

is of course directly adjacent to the eminentia thalami and Professsor Herrick has apparently thought that more was to be included in the unevaginated primordium hippocampi than the writer intended. This structure is the continuation of the fimbria-border of the hemisphere into the dorsal border of the telencephalon medium until it meets the eminentia thalami. This small remnant of the primordium is seen in a model of the forebrain of Necturus standing vertically rostral to the eminentia thalami and separated from the latter by a groove. Figure 36 will show more clearly the relations of this structure. Deep fibers related to the cells of this unevaginated body enter the hippocampal commissure as already described.

As the evagination proceeds the caudal part of the hemisphere soon extends rostrad beyond the level of the interventricular foramen and the lamina terminalis. This is already true in selachians and in amphibians and reptiles the hemisphere protrudes far rostrally (fig. 37). In selachians, it has already been pointed out that the sulcus limitans hippocampi continues rostrally beyond the laterally placed olfactory peduncle and encircles the rostral wall of the hemisphere to end at the neuroporic recess (figs. 38, 39). This portion of the sulcus was called the medial sulcus limitans hippocampi. As compared with cyclostomes, the condition in selachians has resulted from the continued evagination which has carried the olfactory bulbs far laterad and has brought the greater part of the primordium hippocampi into the roof of the hemisphere. The region of the medial olfactory nucleus has bulged forward beyond the lamina terminalis, and the sulcus which separates the primordium from the medial olfactory nucleus in front of the interventricular foramen in Petromyzon comes in selachians to lie in the medio-rostral wall of the hemisphere. In both classes it runs from the neuroporic recess into the lateral ventricle and marks the line of separation of the same structures. It is properly called the sulcus (or zona) limitans medialis. In amphibians the conditions are essentially the same (fig. 37).

Professor Herrick's view as to the evagination of the hemispheres and the relation of his four columns to them is summarized in the following paragraph ('10, p. 477):

The roof plate and floor plate converge into the lamina terminalis, where of course they end. The four massive columns on each side converge into the interventricular foramen, and in larvae with wide foramina and adult urodeles they may be followed through the foramina into the evaginated hemispheres. Bearing in mind the fact that during development the roof plate and floor plate retain permanently their primitive attachments to the lamina terminalis, and that it is only the massive lateral columns which are evaginated into the hemispheres, it clearly follows that these columns of the diencephalon are continued into the hemispheres in the form shown by the accompanying diagram (fig. 84), the zona limitans lateralis representing the locus of the sulcus medius and the zona limiants medialis the line of union of the dorsal and ventral columns in the lateral evaginations rostral to the fusion of the roof plate and floor plate in the lamina terminalis.

It has been noted elsewhere ('11 b, p. 540) that the assignment of the lamina terminalis in this paragraph to the floor plate was an error of inadvertance. It has been shown also ('11 b, pp. 534, 535 and in this paper) that the sulcus diencephalicus medius has no relation with the interventricular foramen or the zona limitans lateralis either in amphibians, fishes or cyclostomes.

The conception of the folding over of the lateral walls of the diencephalon illustrated in Professor Herrick's figures 83 and 84, such that the dorsal and ventral borders fuse in the medial zonae limitantes to form two tubes extending forward (the hemispheres), seems to the writer to be without basis in fact.

In figure 72 Herrick ('10) has given a diagram of the forebrain in a hypothetical vertebrate ancestor. In this the representation of the sulcus dorsalis is subject to the criticisms made above (p. 352). No ground is given for the assumption that the retinal area does not involve the dorsal border of the brain, except the erroneous supposition that there is no di-telencephalic fissure in cyclostomes. The sulcus medius is represented as a horizontal sulcus extending caudad from the site of the interventricular foramen, whereas in all lower vertebrates it runs nearly vertically up into the dorsal sac. The sulcus ventralis should lead to the site of the interventricular foramen as it does in cyclostomes, selachians and amphibians. The terminal ridge is represented as an independent thickening rostral to the chiasma ridge, whereas the terminal ridge in all vertebrate embryos itself becomes the bed for the optic chiasma (Johnston '09).

SUMMARY

The evagination of the hemispheres is at a low stage in petromyzonts. In Ichthyomyzon and Lampetra the formatio bulbaris is not all evaginated. The fact that a part of the wall of the third (median) ventricle in these forms is made up of formatio bulbaris ought to be convincing evidence, if any further evidence is needed, that a part of the third ventricle belongs to the telencephalon. A large part of the secondary olfactory centers is evaginated, but a relatively much larger portion of these centers remains in the telencephalon medium than in the case of selachians. The primordium hippocampi remains wholly in the telencephalon medium. The exact relations of the somatic area await further study.

The di-telencephalic boundary is marked by the attachement of the velum transversum to the eminentia thalami as in selachians ganoids and amphibians. The eminentia thalami is a well-marked ridge just rostral to the nucleus habenulae, bounded by the sulcus medius and by the sulcus limitans hippocampi, and traversed by the stria medullaris.

The telencephalon medium, which is relatively larger than in higher vertebrates, stands as a wedge-shaped mass between the hemispheres. Its massive nervous walls are connected with one another by the lamina terminalis, lamina supraneuroporica and tela chorioidea. The membranous roof plate (His) in the telencephalon of petromyzonts connects the two simple unevaginated lateral walls of the neural tube as in any other segment. The hemispheres are evaginations of restricted areas of these lateral walls. The foramen is roofed over by the massive primordium hippocampi and there is no extension of the tela chorioidea into the roof of the hemispheres.

The primordium hippocampi is represented by the 'praethalamus' of previous authors (the epistriatum of my earlier paper, '02 a). It is a large body having a highly developed and characteristic finer structure which already presents most of the relations of the primordium hippocampi of higher forms (see text). The two primordia converge forward to form a bed for the anterior pallial commissure in the lamina supraneuroporica. The primordium is separated from the epithalamus, thalamus, hemisphere and the

medial olfactory nucleus by a sulcus limitans hippocampi. Along the line of this sulcus is a sudden change of structure which may be compared with the zona limitans of fishes and amphibians.

In vertebrates above cyclostomes the primordium hippocampi is gradually evaginated into the hemisphere, the process being complete in the reptiles. It is only as the hippocampus is carried into the medial border of the hemisphere that the tela chorioidea comes to form the roof of the interventricular foramen and to extend into the roof of the hemisphere.

The expansion of the hemisphere carries both the primordium hippocampi and the medial olfactory nucleus rostrad beyond the lamina terminalis where they form the medial hemisphere wall. The sulcus limitans hippocampi which separates these two nuclei in the telencephalon medium in petromyzonts becomes the sulcus limitans medialis hippocampi in the hemisphere of selachians, amphibians and reptiles.

The relation of the primordium hippocampi to the lamina supraneuroporica in which the anterior pallial commissure lies is fundamental in vertebrates and must be taken into account in seeking the interpretation of higher brains.

The reader is referred to the extended discussion of forebrain morphology contained in the writer's paper on selachians ('11 a, p. 37, especially pp. 47–52). The features of the evolution of the forebrain upon which the cyclostome brain throws light especially are briefly the following.

(a) The hypertrophy, rising dorsad and eversion of the olfacto-gustatory correlation center. The same thing occurs in the visceral receptive column in the medulla oblongata of many fishes and the nucleus habenulae commonly.

(b) The presence originally of the whole olfactory central apparatus in the wall of the medial ventricle. Hemispheres are formed by the evagination first of formatio bulbaris, then secondary olfactory centers, and last the visceral and somatic correlating centers which furnish the materials for the development of the cortical structures.

(c) From a condition like that of cyclostomes the selachian telencephalon has been derived by the evagination chiefly of the

medial olfactory nucleus and the primordium hippocampi. In (Chimaera), ganoids and teleosts the evagination has been arrested and the eversion of the primordium hippocampi seen in cyclostomes is greatly increased. The formatio bulbaris actually bounds the median ventricle near the foramen and the apparent folding out of of the striatal or somatic area in cyclostomes is absent in ganoids and teleosts (see '11 b).

(d) The primitive relations of the hippocampal primordium and of its commissure in the lamina supraneuroporica are splendidly clear and instructive in cyclostomes. The selachians present essentially the same relations in evaginated brains. In ganoids and teleosts ('11 b) the extreme eversion has abolished the primitive supraneuroporic commissure, except in a few forms. In the amphibians the commissures are similar to those of ganoids. In reptiles and mammals the cyclostome and selachian condition reappears.

(e) The view of the general morphology of the telencephalon previously expressed ('10 c, '11 a, '11 b) receives the strongest support from these most primitive brains. The functional columns of the brain described by the writer ('02 b) extend forward to the telencephalon. The ventral columns are represented only by correlating tissue adjacent to the terminal ridge or optic chiasma. The dorsal columns constitute the overwhelming part of the telencephalon, are greatly hypertrophied and become flexed in consequence. The dorsal and ventral columns meet in the preoptic recess where the sulcus limitans of His ends. The telencephalon in petromyzonts is not elongated parallel with the long axis of the fish, but owing to its flexure, is longer dorso-ventrally.

The olfactory nerve and sac on the one hand and the formatio bulbaris on the other are closely related to the neuroporic recess and illustrate clearly the principle ('11 a, p. 39) that the neuroporic recess owes its existence to the attachment of the olfactory nerve to the dorsal lip of the neural tube at this point. The evagination of hemispheres began here close to the dorsal border at some distance from the anterior end of the brain tube. This point lies at about the middle or height of the forebrain flexure.

Caudal to this middle point of the forebrain the visceral receptive column forms the olfacto-gustatory correlation center or primordium hippocampi. From the study of other fishes ('11 a and '11 b) I have shown reason to think that the somatic correlation center, or primordium of the somatic cortex has been formed by eversion and migration of neuroblasts from the dorsal border of the telencephalon in this caudal part.

The cephalic part of the forebrain comes to be ventrally placed owing to the flexure, and is constituted chiefly or wholly of the medial and lateral secondary olfactory centers and preoptic nucleus. The formatio bulbaris arises from tissue nearest the olfactory nerve root at the middle part of the dorsal column and in higher forms is carried out upon a peduncle. In some petromyzonts it actually retains its relation to the median ventricle.

An understanding of the fundamental morphological and functional relations in the forebrain must be built upon the central fact of the forebrain flexure. The conception of a curved axis of the telencephalon ending at the preoptic recess, and of the olfactory nerve entering the dorsal border at the height of the curve must never be lost sight of. These are fundamental facts, not hypotheses, and all forebrain morphology becomes confused unless these facts are held firmly in mind.

In forms above the cyclostomes the rostrum elongates, the olfactory sac shifts forward, the bulbar formation follows it and becomes pedunculated. At the same time the evagination of hemispheres progresses rapidly and the hemispheres elongate. Now the cephalic and caudal portions of the visceral receptive column are sharply flexed on one another in the form of a letter *U* (figs. 40, 41). The olfactory tract enters the curve of the *U*. The cephalic limb, containing the secondary olfactory centers is ventrally placed and ends at the preoptic recess. The caudal limb (primordium hippocampi) is dorsally placed and ends at the eminentia thalami. The two limbs are separated by the interventricular foramen and by the medial and lateral zona limitans as already explained.

Owing to the eversion and migration of the neuroblasts of the somatic correlating center down upon the lateral surface of the telencephalon this primordium of the general cortex comes to lie

between the limbs of the *U*. In this position it enters into relations with the lateral olfactory nucleus which lead to the formation of the pyriform lobe as an olfacto-somatic correlation center (cortex). Within the somatic primordium there develop also complex correlation systems between the cutaneous, musculo-sensory, auditory and visual fiber tracts which probably reach this center at an early stage in the phylogeny (see '10 c, and '11 a). This complex correlating organ is the general cortex. As it develops it pushes in between the primordium hippocampi and the pyriform lobe, assuming the characteristic postion of the general cortex in mammals. The expansion of the general cortex pushes the hippocampal formation to the medio-dorsal border of the hemisphere and the pyriform lobe down to the ventral surface.

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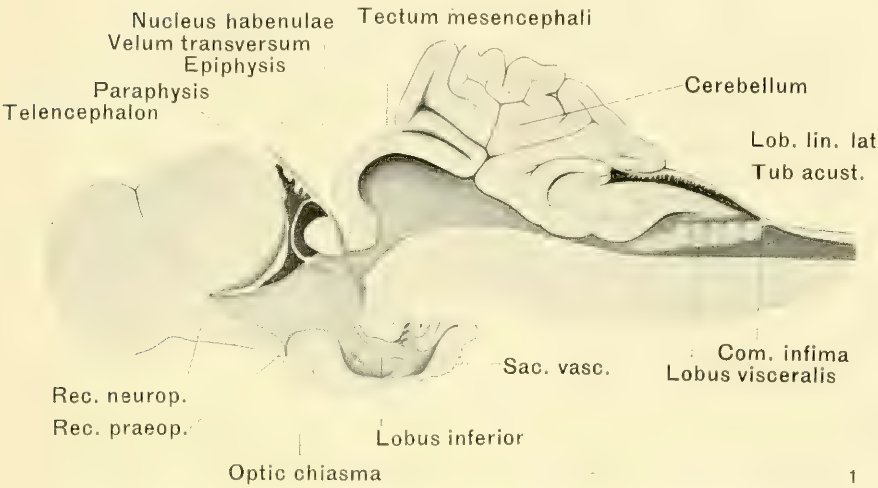
REFERENCE LETTERS

b.o., bulbus olfactorius, formatio bulbaris.
c. a., commissura anterior
ch., chiasma opticum.
c.hipp., commissura hippocampi.
c.p., commissura posterior.
c.p.a., commissura pallii anterior.
c.p.p., commissura pallii posterior.
c.sup., commissura superior (Osborn).
dienc., diencephalon.
di-tel., di-telencephalic boundary.
d.p., decussatio postoptica.
e., epiphysis.
em.th., eminentia thalami.
f.i., foramen interventriculare.
f.olf., fila olfactoria.
f.p.p., fibers from the parapineal body.
g., olfactory glomeruli.
hem., hemisphere.
hy., hypophysis.
hyp., hypothalamus.
l.o., lobus olfactorius (secondary centers).
l.s., lamina supraneuroporica.
l.t., lamina terminalis.
l.v., lateral ventricle.
m., caudal margin of lamina supraneuroporica.
mesenc., mesencephalon.
n.h., nucleus habenulae.
n.olf., nervus olfactorius.
nuc.olf.med., nucleus olfactorius medialis.
par., paraphysis.
p.c.b., precommissural body.
p.h., primordium hippocampi.
p.p., parapineal body.
pt., pretectal region.
rec.ph., recessus praehabenularis.
r.m., recessus mammillaris.
r. n.,recessus neuroporicus.
r.p., recessus praeopticus.
r.po., recessus postopticus.
s.d., saccus dorsalis.
s.l., sulcus limitans hippocampi.
s.l.H., sulcus limitans of His.
s.m., stria medullaris.
s. m. 1, first bundle of stria medullaris
s.med., sulcus diencephalicus medius of Herrick.
s.hy., sulcus hypothalamicus.
sub.hab., subhabenular region.
t.c., tela chorioidea.
tel.m., telencephalon medium.
t.f., taenia fornicis.
thal., thalamus.
t.p., tuberculum posterius.
tr.c-h., tractus cortico-habenularis.
tr.h-p., tractus habenulo-peduncularis.
tr.o-h., tractus olfacto-habenularis.
tr.op., tractus opticus.
tr. pall.,tractus pallii.
v. tr., velum transversum.

Fig. 1 The medial surface of the right half of the brain of Mustelus to show the position of the velum transversum in selachians. It is attached to a slight eminentia thalami which is not lettered.

Fig. 2 Medial view of the right half of the telencephalon and diencephalon of an adult Acipenser. The olfactory bulb is not drawn. The velum transversum is a very deep fold of the tela chorioidea which is nearly horizontal in position. It is attached to the eminentia thalami as in selachinas, amphibians and others.


Nucleus habenulae
Velum transversum
Epiphysis
Paraphysis
Telencephalon
Tectum mesencephali
Cerebellum
Lob. lin. lat.
Tub acust.
Com. infima
Lobus visceralis
Sac. vasc.
Rec. neurop.
Rec. praeop.
Lobus inferior
Optic chiasma

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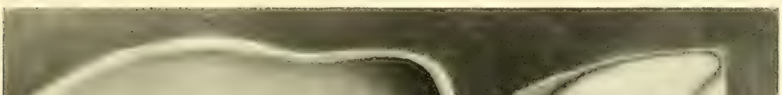
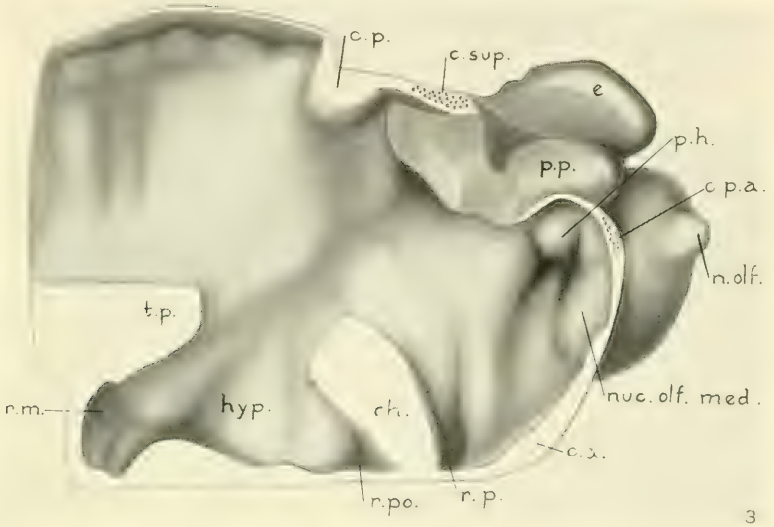

saccus dors.
valvula
vel. transv.
prim.
hipp.
g.hab.
com. ant.
hypothal.
2

Fig. 3 Petromyzon dorsatus, ammocoetes. Medial view of a model of the left half of the forebrain.

Fig. 4 Lateral view of the model shown in figure 3. The hemisphere is divided by a vertical sulcus into a rostral portion containing the bulbar formation and a caudal portion containing the secondary olfactory centers. About opposite the caudal pole of the hemisphere appears a vertical sulcus in the wall of the brain stem. This corresponds in position to the di-telencephalic boundary line as determined by other data and is to be regarded as a di-telencephalic sulcus or fissure.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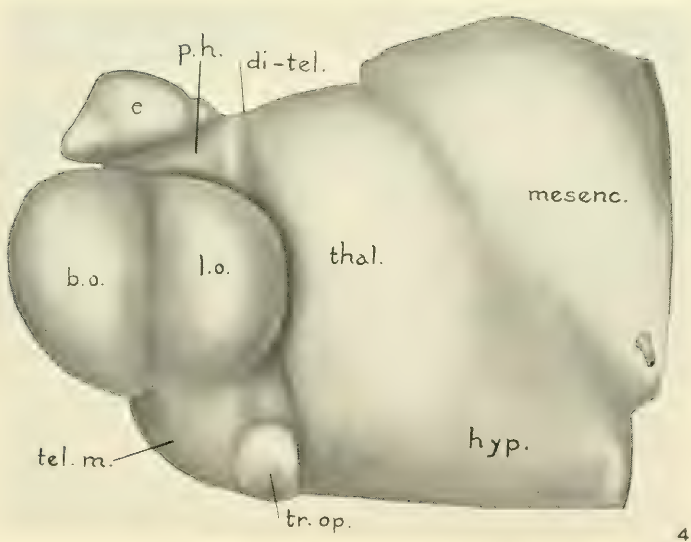
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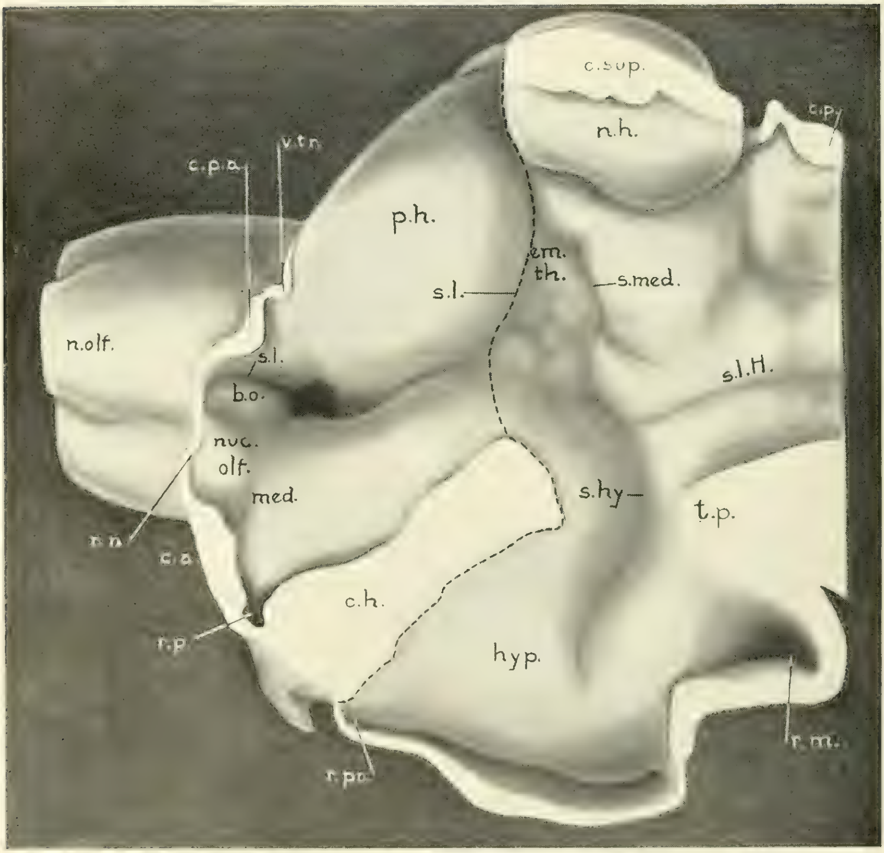


Fig. 5 Lampetra wilderi. Medial view of the right half of a model of the telencephalon and diencephalon. × 50.

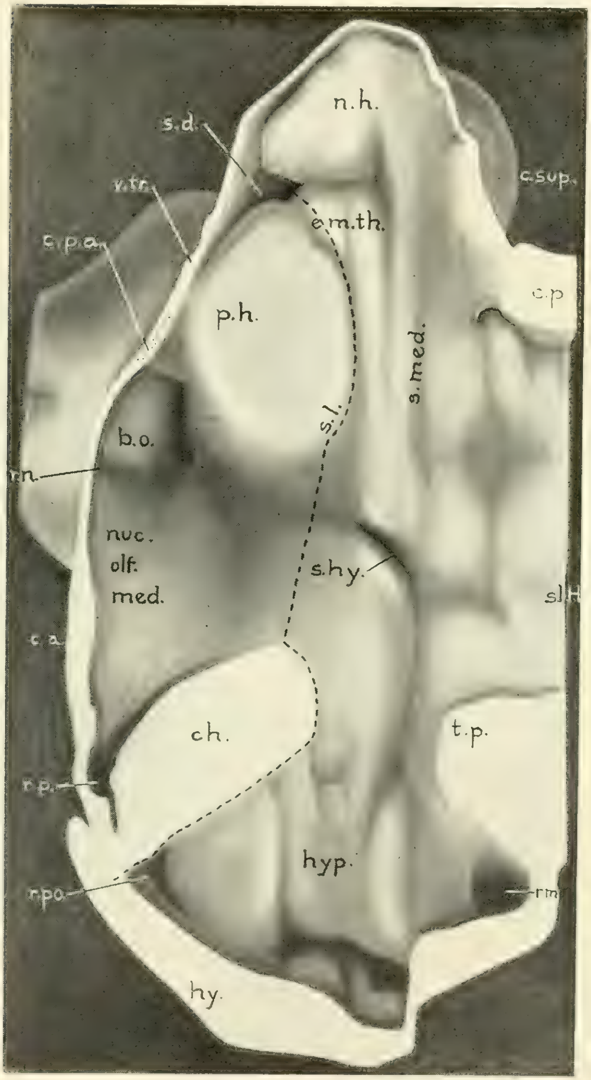


Fig. 6 Ichthyomyzon concolor. Medial view of the right half of a model of the telencephalon and diencephalon. × 66. The slender groove rostral to the sulcus medius is apparently due to one or two sections being less stretched in mounting than the adjacent ones.

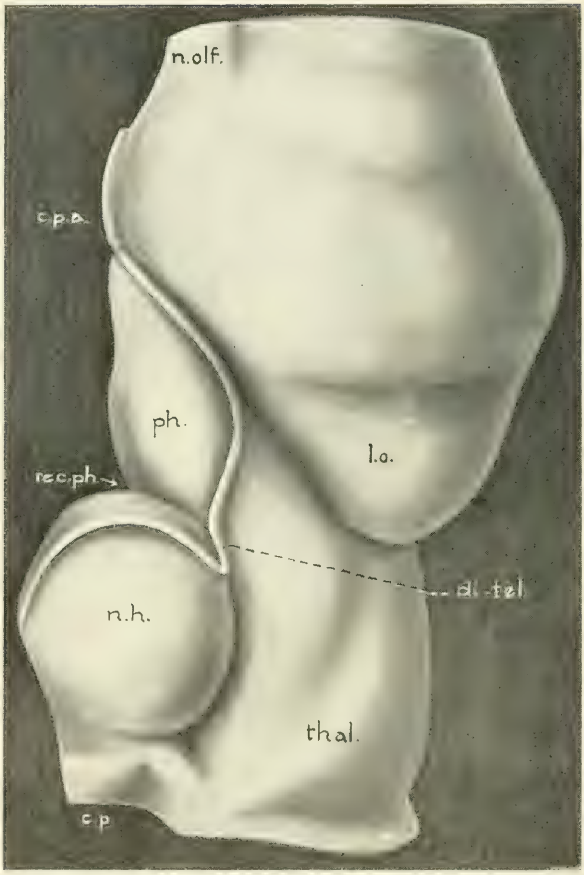


Fig. 7 Lampetra. Dorsal view of the model shown in figure 5. This is presented chiefly to illustrate the stem-hemisphere fissure and the wedge-shaped telencephalon medium. The attached border of the tela chorioidea is drawn. Its caudo-lateral angle is the upper end of the recessus praehabenularis. In Petromyzon dorsatus the eminentia thalami comes up nearly to this point and the velum transversum is attached to it. The broken line running caudo-laterad from this point corresponds to the plane of sudden transition in internal structure from the primordium hippocampi to the pretectal and subhabenular region.

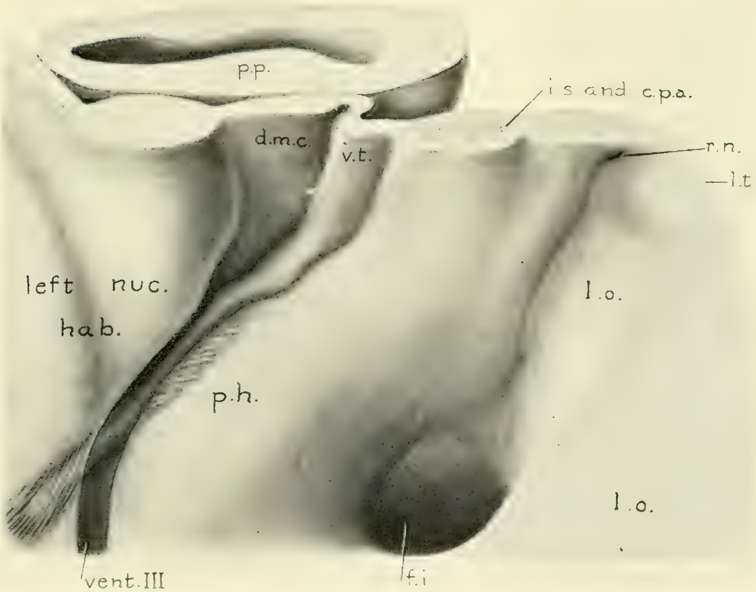


Fig. 8 Petromyzon dorsatus, ammocoetes. Model of the dorsal portion of the left half of the forebrain, drawn as seen from the medio-ventro-caudal direction. The model was carefully constructed on a large scale in order to determine whether the velum transversum really formed a continuous fold from the median line to the lateral attachment of the tela (i. e., the taenia). This drawing should be compared with figures 23 to 27. *d.m.c.*, dorsal sac.

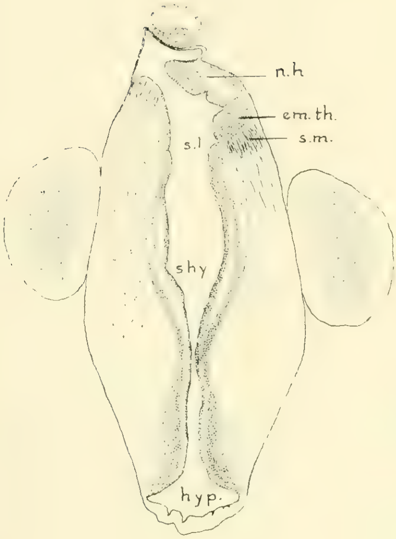
Fig. 9 Ichthyomyzon concolor. A transverse section through the nucleus habenulae and eminentia thalami of the right side. The section cuts the eminentia thalami at its most prominent part, where it is bounded by the sulcus limitans hippocampi below and the sulcus subhabenularis above, as seen in figure 6.

Fig. 10 Petromyzon dorsatus, adult. Transverse section through the interventricular foramen. The primordium hippocampi is not so large as in Lampetra. At the lip of the foramen it is continuous with the wall of the hemisphere.

Fig. 11 Same series as figure 10, section through the eminentia thalami.

Fig. 12 Entosphenus, ammocoetes of 35 mm. Transverse section through rostral part of foramen interventriculare. The section is quite oblique so that on the right side it passes considerably rostral to the foramen. The primordium hippocampi is small but occupies the same position as in other forms. The section falls at the junction of the parapineal body and the left nucleus habenulae.

Fig. 13 Same series as figure 12, section through the eminentia thalami and the habenular nuclei. Note the great size of the right nucleus habenulae. On the right side the primordium hippocampi is cut.



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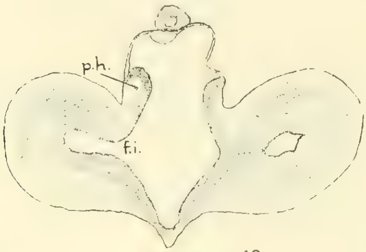

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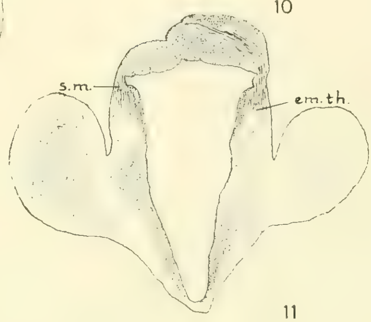

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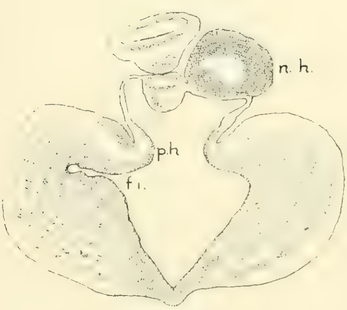

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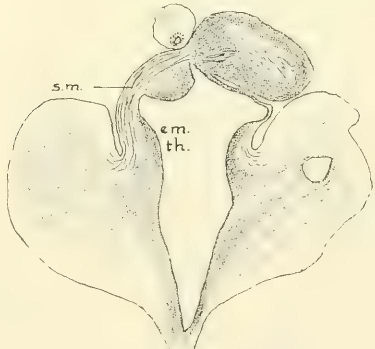
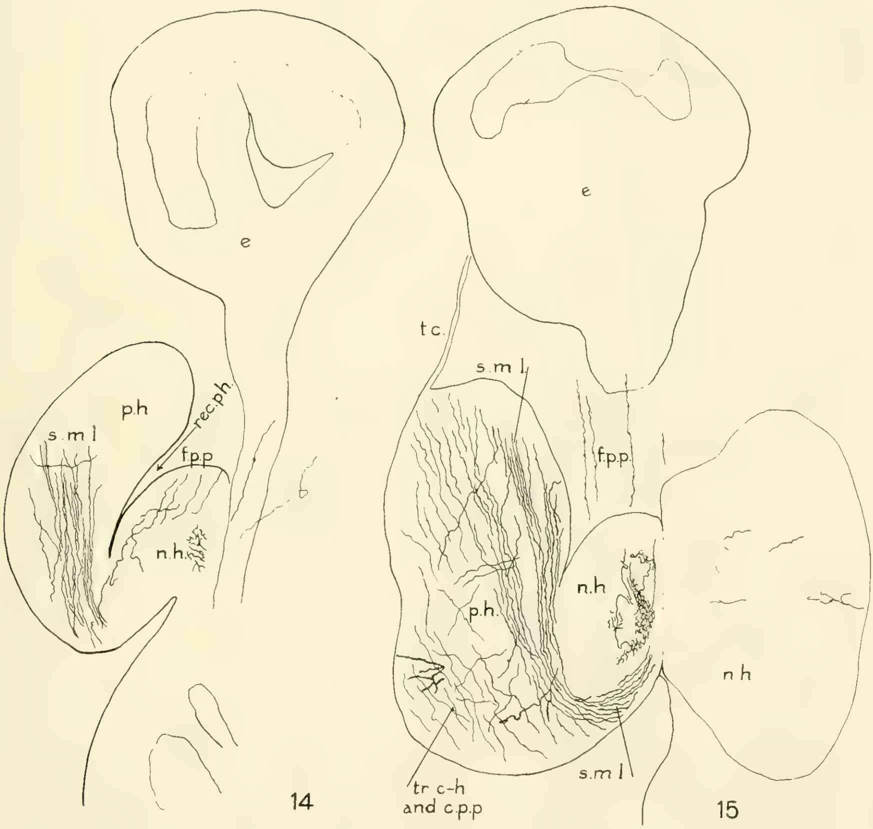


13

Figs. 14–22 Lampetra. Nine sections of the habenular and hippocampal region drawn from a series of horizontal sections prepared by the Golgi method. The figures were drawn at a magnification of 200 diameters and are reduced to one-third. Figure 22 is at a somewhat lower magnification.

Fig. 14 The first section through the dorsal part of the left primordium hippocampi. The section shows the epiphysis and its stalk. The four fibers in the left nucleus habenulae come from the parapineal body. The bundle *s.m.1* is the first part of the stria medullaris of which it is uncertain whether it is a tractus olfacto-habenularis or tractus cortico-habenularis.

Fig. 15 The second section of the same series. The first bundle of the stria medullaris is passing through the left nucleus habenulae.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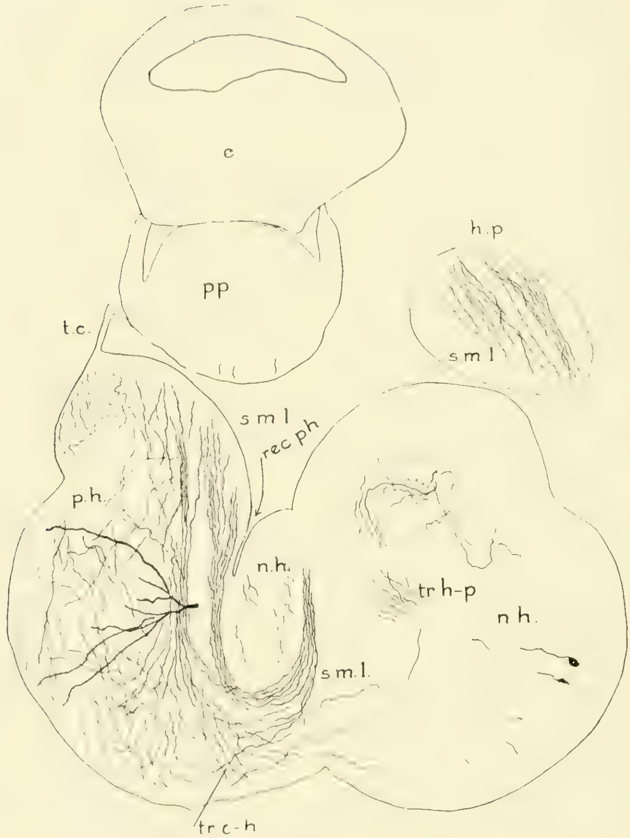


Fig. 16 The third section of the same series. Note the extreme difference in size between the two nuclei habenulae and the passage of the first stria medullaris fibers as a compact bundle over to the right nucleus. The prehabenular recess is narrow in Lampetra and the eminentia thalami does not appear as a distinct ridge in these dorsal sections. This is due to the large size of the primordium hippocampi in Lampetra as well as to the crowding together on account of the pressure of the buccal funnel. A section through the brain of Ichthyomyzon or Petromyzon dorsatus at this same level would show a distinct eminentia thalami. Compare figures 6 and 9.

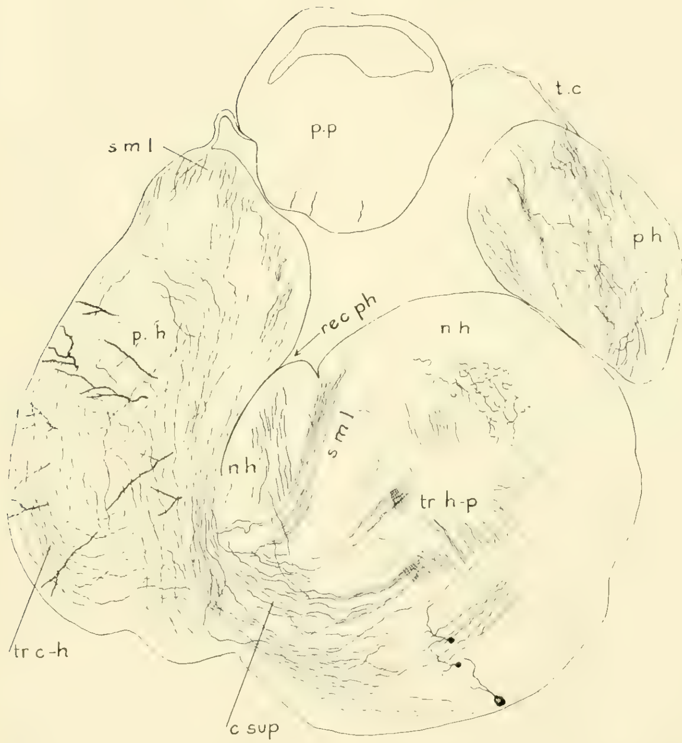


Fig. 17 The fourth section of the same series. Note the several divisions of the commissura superior (Osborn). The next three figures show that the great majority of the left stria medullaris decussates and that a large number of fibers pass through the nuclei habenulae to the opposite primordium hippocampi.

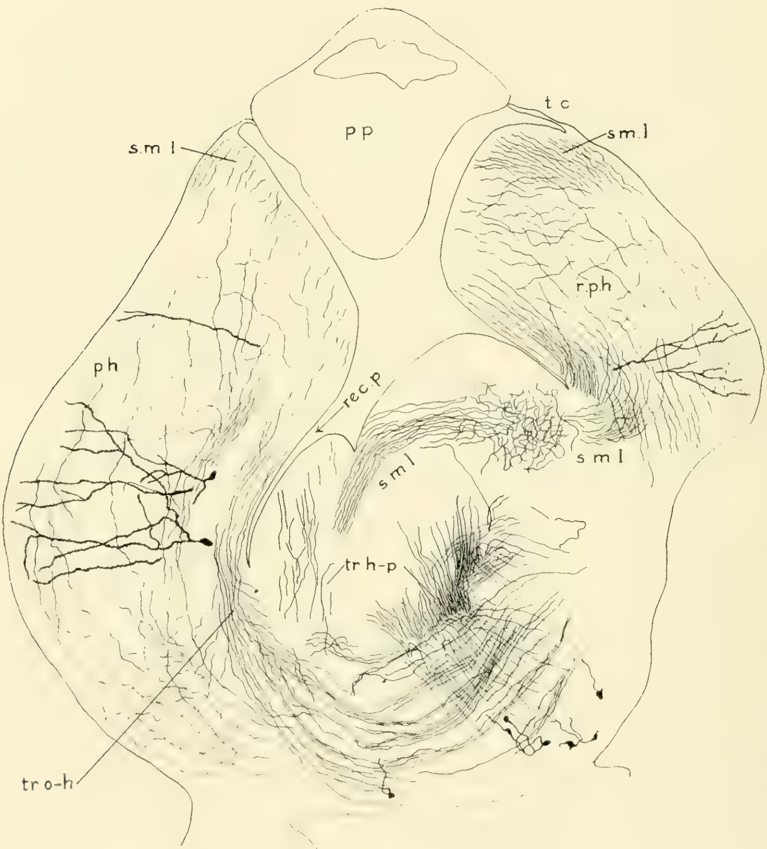


Fig. 18 The fifth section of the same series. The right primordium hippocampi shows the first stria medullaris bundle. On the left the tractus olfacto-habenularis comes up through the lower part of the primordium hippocampi and the eminentia thalami.

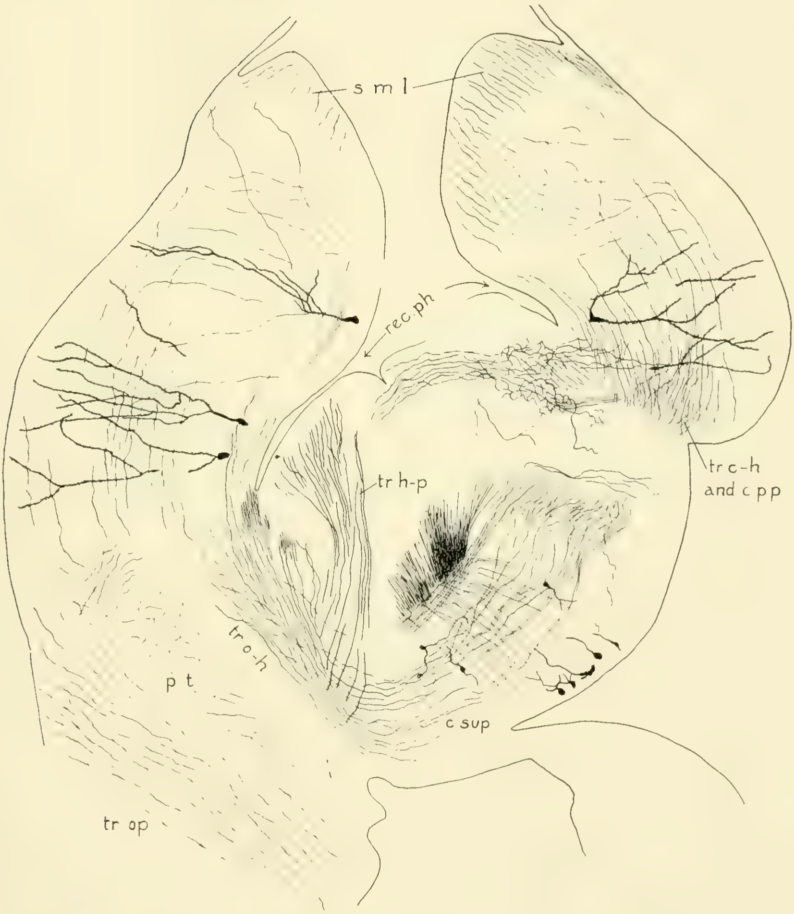


Fig. 19 The sixth section of the same series. On both sides the fibers of the tractus olfacto-habenularis lateralis and posterior appear as definite bundles which in the next sections clearly lie in the eminentia thalami.

Fig. 20 The seventh section of the same series. In the rostral part of the right primordium hippocampi is seen a cell whose axone is directed toward the nucleus habenulae. Note the sharpness of the structural boundary between the primordium hippocampi and the pretectal region.

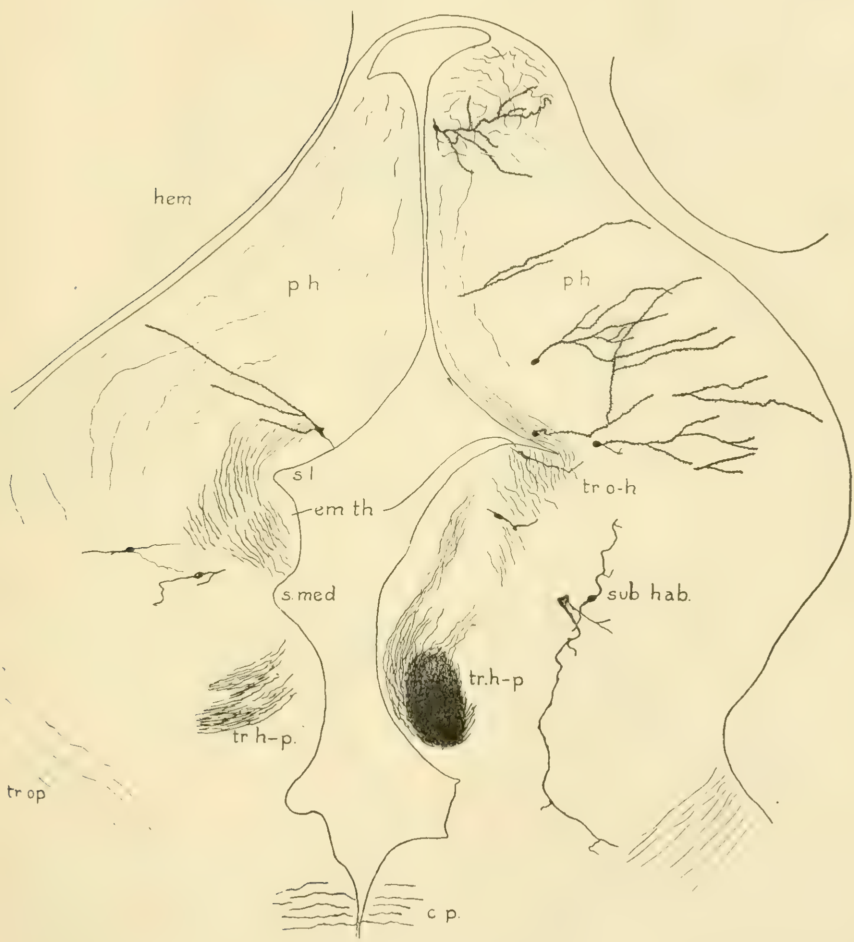


Fig. 21 The ninth section of the same series. Note the difference between the cells in the primordium hippocampi and those in the subhabenular region. The tractus olfacto-habenularis occupies a position in the eminentia thalami which is quite typical for vertebrates generally. The great number of longitudinal fibers in the primordium hippocampi are only indicated by a few lines.

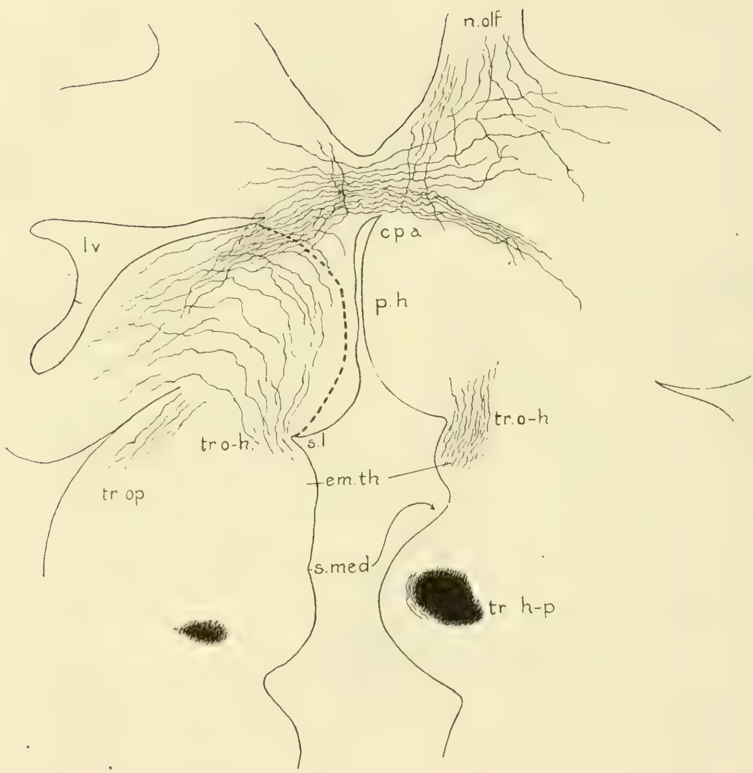
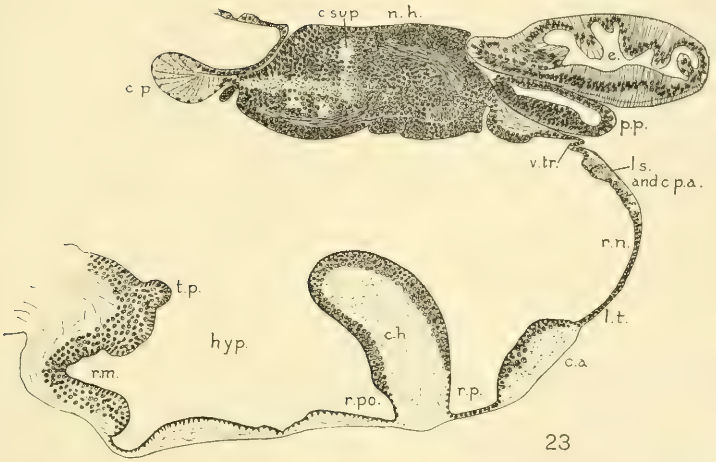


Fig. 22 The thirteenth section of the same series drawn at a lower magnification. The section passes through the anterior pallial commissure and above the interventricular foramen. The heavy broken line shows the outline of the next section ventrad, in which the sulcus limitans hippocampi passes forward to enter the foramen. Note that the primordium hippocampi is followed caudally by the eminentia thalami and this by the ridge containing the tractus habenulo-peduncularis. The sulcus medius separates these two ridges. The anterior pallial commissure in this section has only fibers connecting the hemispheres.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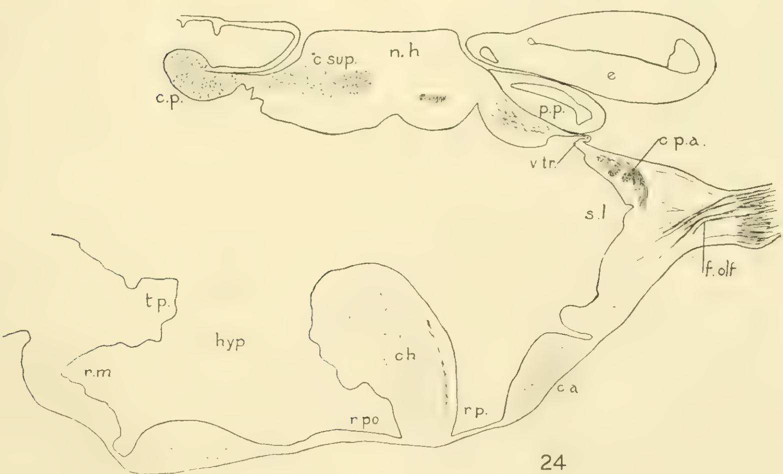
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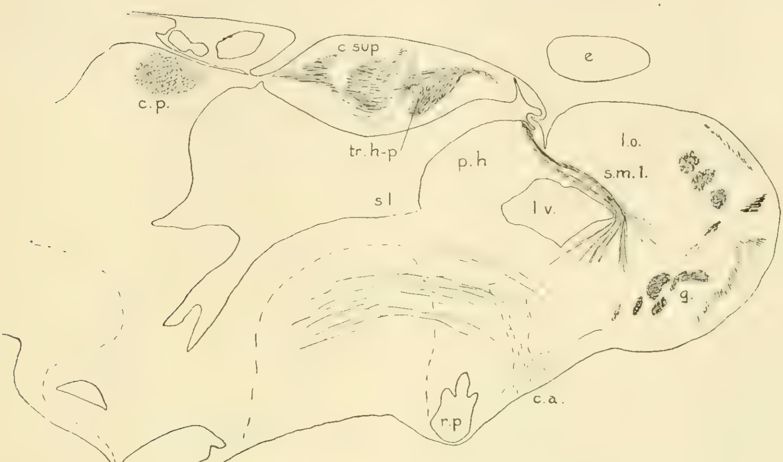
Figs. 23–27 Ammoecoetes of Petromyzon dorsatus. Five sections from the sagittal series from which the model shown in fig. 8 was reconstructed. The outlines were made with the aid of the Edinger drawing apparatus. The velum transversum is seen as a distinct fold of the tela in figures 23 to 26, but in figure 27 it joins the eminentia thalami. Figures 25 and 26 show well the course of the tract which runs up from the medial olfactory nucleus in front of the foramen interventriculare. This bundle may contain a tractus olfacto-corticalis and possibly also the fornix column. The fibers of the commissural bundles beginning with the most rostral successively turn up into the primordium hippocampi (fig. 24). The fibers which go laterally into the hemispheres are not readily seen in these sagittal sections because they are cut across. These sections show conclusively that the commissure is largely related to the primordium hippocampi.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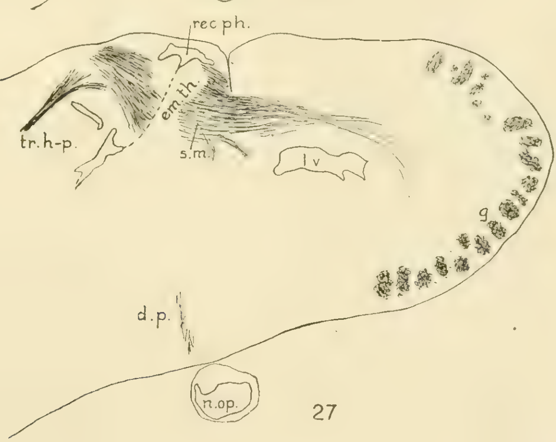
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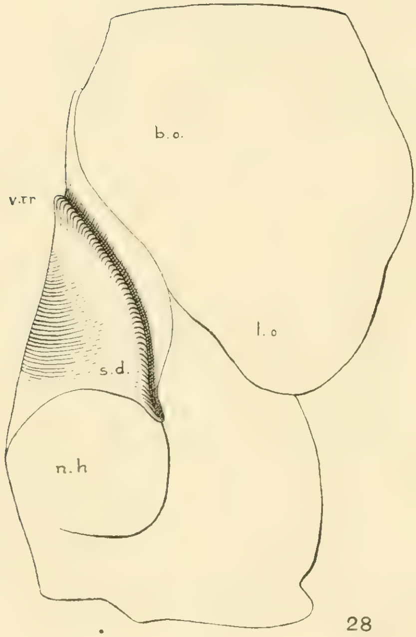

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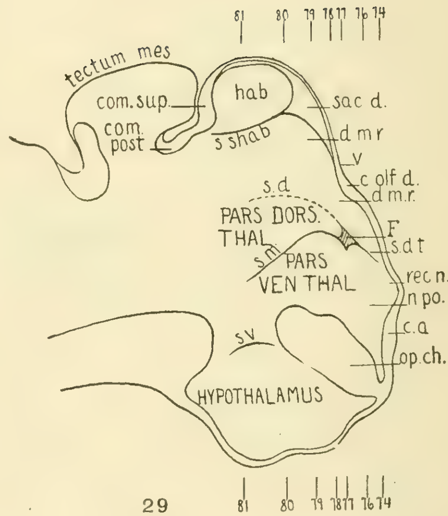

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Fig. 28 A diagram to show the disposition of the velum transversum. The outline is taken from figure 7, showing the dorsal aspect of the model of the Lampetra brain. The tela chorioidea is drawn as it would appear if the epiphysis and parapineal body were removed. The velum transversum is drawn as a fold of the tela.

Fig. 29 Professor Herrick's figure 73 reprinted for comparison with figures 6 and 9 of this paper. The figure is "a diagram reconstructed from actual sections of the cyclostome brain, Ichthyomyzon concolor."

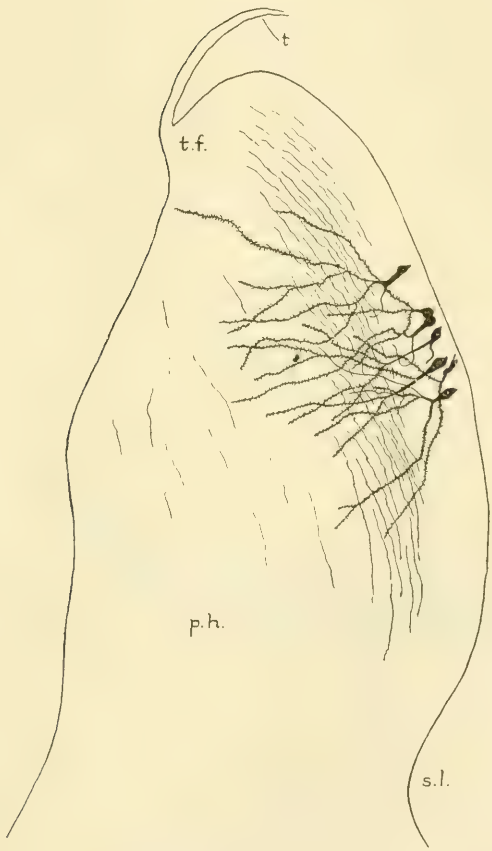


Fig. 30 Lampetra. Transverse section through the caudal third of the left primordium hippocampi. Golgi method. Most of the dendrites are longer than they appear in this figure. The distal ends of the dendrites extend nearly to the outer surface but are too intricately interlaced to be followed.

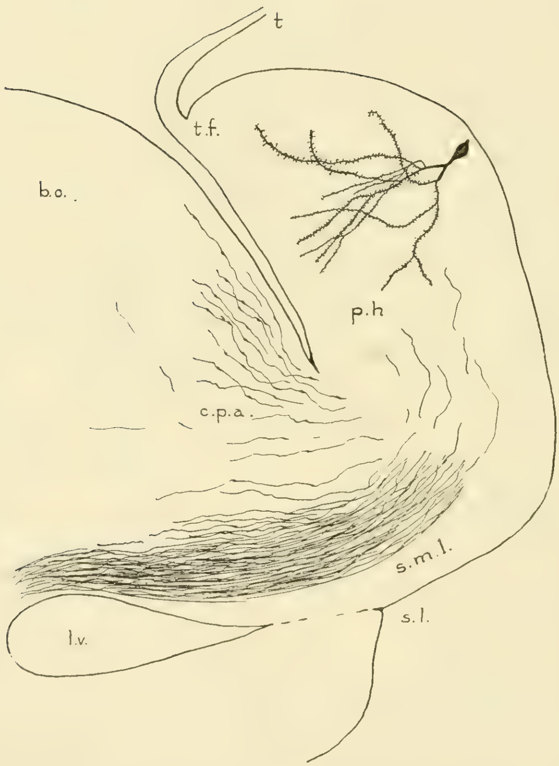


Fig. 31 Lampetra. Transverse section through the primordium hippocampi just caudal to the interventricular foramen and the anterior pallial commissure. The cells in the rostral end of the primordium are of the same type as in the caudal part (fig. 30).

Fig. 32 Acipenser rubicundus. Five cells of the primordium hippocampi for comparsion with those of Lampetra. Note that the eversion of the forebrain in this ganoid of 30 cm. is about the same as in the adult Lampetra.

Fig. 33 Amia calva, about 15 mm. A parasagittal section near the lateral border of the forebrain, showing three cells of the primordium hippocampi and a few fibers of the tractus pallii. Golgi method.

Fig. 34 Rana. Transverse section of the dorso-medial angle of the right hemisphere showing the characteristic cells of the primordium hippocampi. Golgi method.

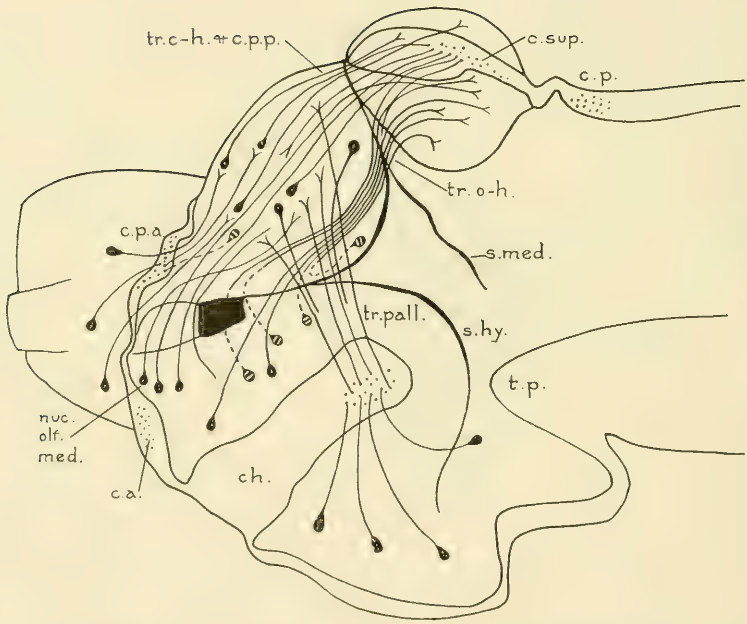
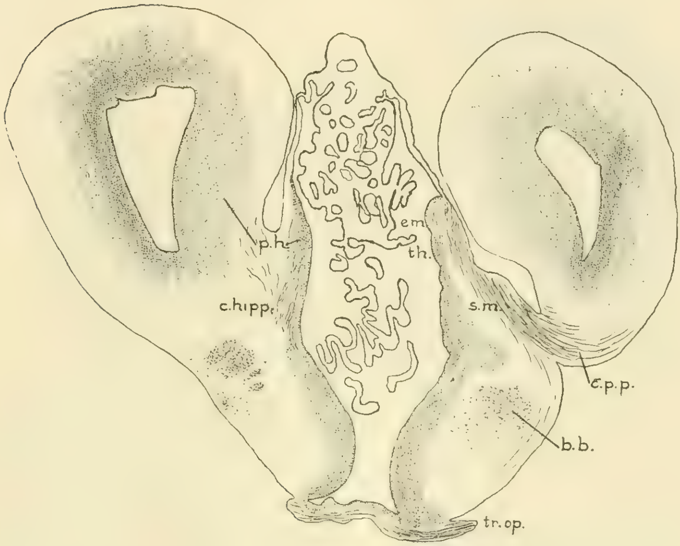


Fig. 35 Scheme of fiber tracts related to the primordium hippocampi in petromyzonts. The secondary olfactory neurones located in the caudal part of the hemisphere are represented by broken lines.



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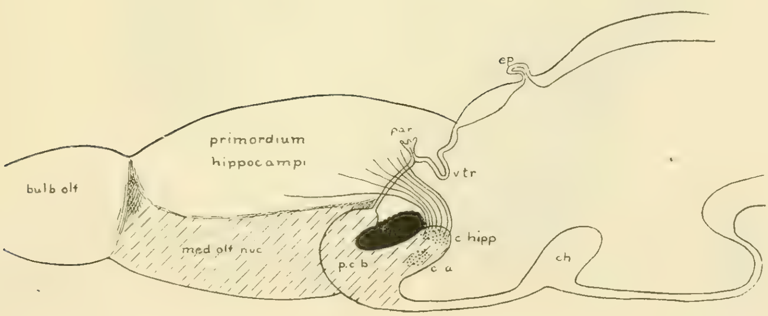

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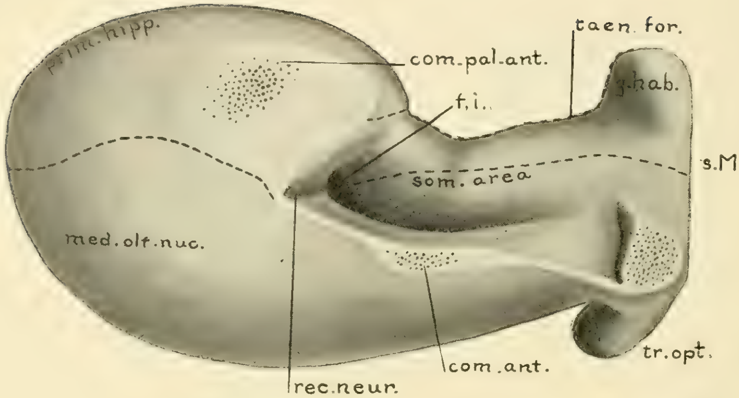
Fig. 36 Cryptobranchus allegheniensis. Transverse section through the telencephalon behind the cerebral commissures. The remnant of the primordium hippocampi in the telencephalon medium appears on the left side to the right of the letters *p.h.*

Fig. 37 Necturus. Sketch of the right half of the forebrain as seen from the medial surface. This figure is introduced merely to show the general relations of the medial zona limitans which extends rostrally from the interventricular foramen and separates the medial olfactory nucleus and primordium hippocampi. Its formation has been brought about by the evagination of the region which in the brain of cyclostomes lies rostral to the foramen. Compare figure 5.



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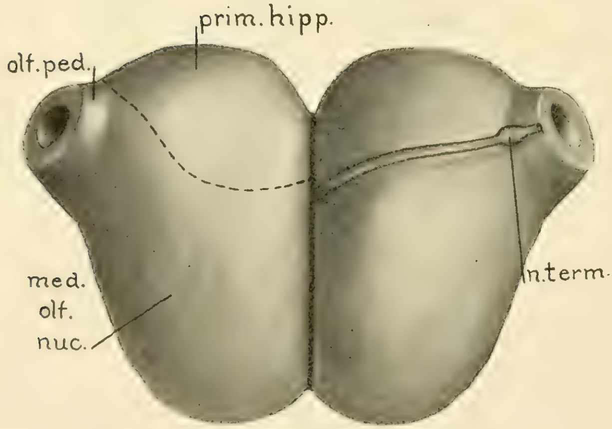

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Figs. 38 and 39 Two drawings of a clay model made to represent a simplified selachian forebrain. From Johnston '11 a. Figure 38 shows the medial surface, figure 39 the rostral surface. The broken line on these surfaces represents the medial zona limitans hippocampi. It extends from the neuroporic recess through the interventricular foramen along the medial wall of the hemisphere. Compare the figures of sections in the paper referred to. This zona limitans is homologous with the line of separation of the primordium hippocampi and medial olfactory nucleus rostral to the foramen in cyclostomes. See figures 3, 5 and 6. In figure 38, *s.M.* marks the continuity of the sulcus limitans hippocampi with the sulcus hypothalamicus, very much as in Lampetra and Ichthyomyzon. Compare figure 41.

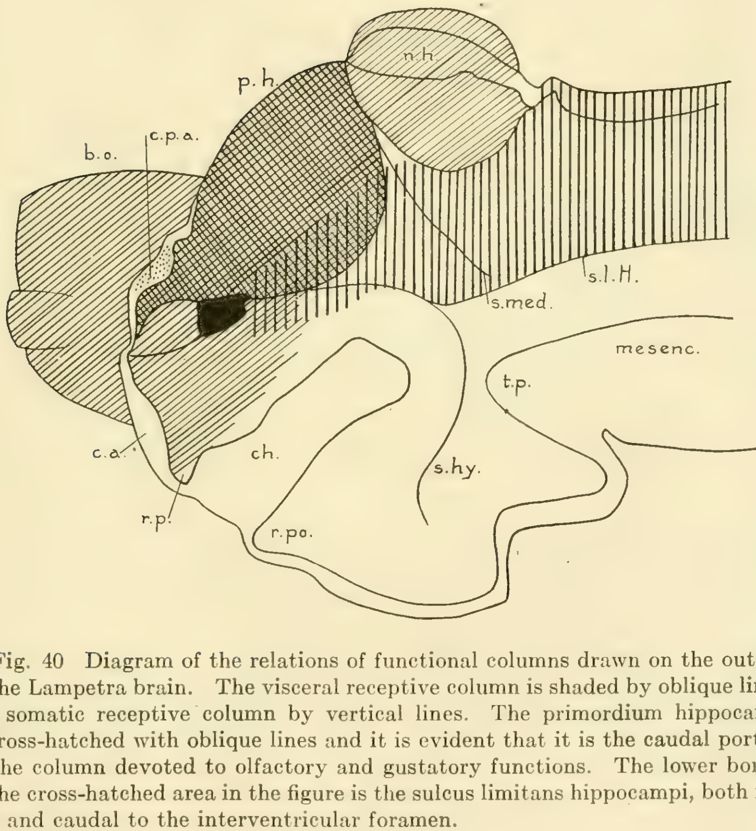

Fig. 40 Diagram of the relations of functional columns drawn on the outline of the Lampetra brain. The visceral receptive column is shaded by oblique lines, the somatic receptive column by vertical lines. The primordium hippocampi is cross-hatched with oblique lines and it is evident that it is the caudal portion of the column devoted to olfactory and gustatory functions. The lower border of the cross-hatched area in the figure is the sulcus limitans hippocampi, both rostral and caudal to the interventricular foramen.

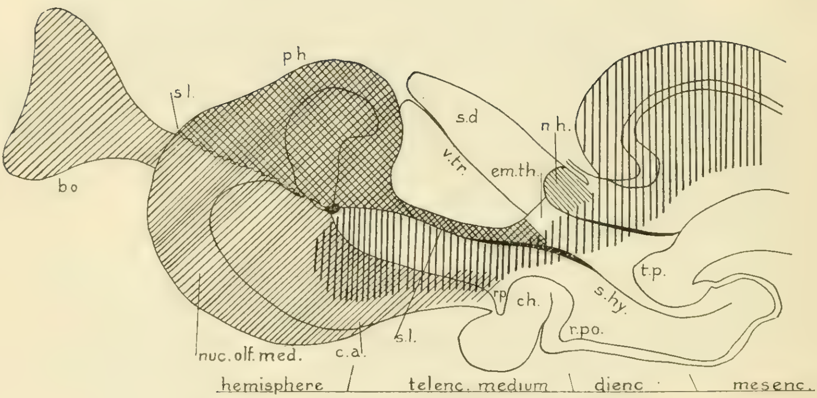

Fig. 41 Diagram similar to that of figure 40, drawn on the outline of the brain of Squalus acanthias. Drawn from the bisected brain. Owing to the somatic receptive area being situated on the lateral surface of the telencephalon, its projection on this figure overlaps the olfactory area. The medial sulcus limitans hippocampi is marked by a wavy line along the lower border of the primordium. This figure illustrates the U-shaped flexure of the visceral receptive column, the attachment of the olfactory peduncle at the base of the *U*, and the position of the somatic receptive area between the two limbs of the *U*. The axis of the brain ends in the recessus praeopticus and the great forebrain flexure involves only the dorsal columns.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE, NO. 234

THE NUMERICAL RELATIONS OF THE HISTOLOGICAL ELEMENTS IN THE RETINA OF NECTURUS MACULOSUS (RAF.)

SAMUEL C. PALMER

TWELVE FIGURES

CONTENTS

I. INTRODUCTION

Although the vertebrate eye has long attracted the attention of investigators and has been the subject of numerous researches, few attempts to enumerate the histological elements of the retina and of the optic nerve have been made. The exceedingly delicate nature of the retina has always been a serious difficulty in the way of its histological study, but with the introduction

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of Golgi's bichromate method and of Ehrlich's methylene-blue method for staining nervous tissues, this difficulty has been, at least partially, overcome, and as a result great advance has been made in our knowledge of the visual apparatus. We owe much, also, to the use of osmic acid, which has been so successfully employed, not only in the study of the finer structure of nerve cells and their processes, but also in the enumeration of nervous elements. In the optic nerve this has not always been satisfactorily carried out, because the fibers of this nerve are very small and difficult to stain. The usual treatment has been to blacken the medullary sheaths with osmic acid, which, however, leaves the axis-cylinders unstained.

Many theories have been elaborated to explain the structure and functions of the retinal elements, but where the numerical relations of these units have been concerned investigators have, for the most part, confined their enumerations to regions of special interest, leaving untouched the broader aspect of the entire retina and optic nerve. It has seemed to me, therefore, that a complete enumeration of the histological elements of the retina and of the optic nerve, which have to do directly with the transmission of visual impulses, would be a desirable addition to our knowledge of the eye; and I have, therefore, undertaken to complete such an investigation on one of the lower vertebrates.

There is great similarity in the structure of the retina in all classes of vertebrates. From the standpoint of the neurone theory, the visual apparatus in these animals consists of three distinct layers of neurones. These neurones bear so constant a relation to one another that a definite terminology has become associated with them. The first neurones to receive the visual impulses are the visual cells, which include the rod-and-cone layer, a majority of the nuclei in the outer nuclear layer, and processes terminating in the outer reticular layer. The second set of neurones is represented by the nuclei of the inner nuclear layer exclusive of Müller's fibers and are of two distinct forms, viz., amacrine cells and bipolar cells. Processes from the latter form synapses with similar processes arising from the visual and from the ganglion cells. The third set of neurones consists of

the ganglion cells, which are described by Ramón y Cajal ('94) as sending free branching processes into the inner reticular layer, and axis-cylinders to the central organ through the optic stalk.

My problem is concerned with enumerations of the rods, cones, and double-cones, and the nuclei associated with them, of the nuclei of the inner nuclear layer, and the ganglion layer, of the Müller's fibers, and the fibers of the optic nerve.

All my investigations have been made in the Zoölogical Laboratory of Harvard University under the personal supervision of Prof. G. H. Parker, to whom I am greatly indebted for advice and valuable criticism.

II. HISTORICAL REVIEW

A. Retina

Although the literature on the retina and optic nerve in vertebrates is extensive, it is surprising that so little has been done on the numerical relations of the retinal elements. For the most part, those who have investigated the retina in this respect have limited their statements to the fovea and to comparisons between the number of visual and ganglion cells in central and peripheral regions. An epitome of the morphology and physiology of the retina has been published by Greeff ('00), but it is not satisfactory for numerical relations of the elements because of the lack of sufficient data.

Much of what has been written concerning the number of retinal elements in mammals relates to the human retina. One of the earliest to consider the question was Krause ('76), who estimated the number of rods in the human retina to be 130,000,000 and of cones to be 7,000,000. Salzer ('80) also worked on the human retina and estimated the number of cones to lie between 3,000,000 and 3,600,000, but gave no estimate of the number of rods or of other retinal elements. Foster ('91) and many other physiologists have apparently accepted the estimates of Salzer. In regard to the distribution of the rods and cones Foster writes "Over the retina (including the ora) the rods are much more numerous than the cones, there being two or three rods

in the line joining two cones,'' and ''Toward the extreme periphery of the retina the cones become more numerous and close to the ora are alone present.'' Hesse ('00) gives the total number of visual cells in the human retina as 50,000,000. Stöhr ('06) believed that the rods were more numerous than the cones and that the latter occurred at regular intervals, so that three or four rods lay between adjacent cones. Pütter ('02) has given a great deal of attention to enumerations of the histological elements in the retina and in the optic nerve of marine mammals. The total number of elements in the different layers of the retina was found to be enormous (table 1). Estimates of the number of rods ranged from 227,000,000 in the adult Phoca vitulina to 800,000,000 in Macrorhinus leoninus and Balaenoptera physalus. As no mention was made of cones, except in the case of Hyperoodon rostratus, where they were said to be wanting, the retinas were presumably pure rod retinas. The nuclei in the outer nuclear layer were said to be from six to thirteen times as numerous

TABLE 1

Estimated numbers of retinal elements and optic nerve fibers in certain species of marine mammals (after Pütter, '02)

SPECIES	NUMBER OF RODS PER SQUARE MILLIMETER	MAXIMUM NUMBER OF RODS IN RETINA	NUMBER OF NUCLEI PER SQUARE MILLIMETER IN		NUMBER OF OPTIC NERVE FIBERS		RATIO OF OPTIC NERVE FIBERS TO RODS
			Outer nuclear layer	Inner nuclear layer	Per square millimeter	Total	
Macrorhinus leoninus	90,000	800,000,000	1,250,000	110,000	103	767,000	1:1050
Phoca barbata	120,000	363,000,000	1,367,000	119,000	68	174,000	1:2086
Phoca vitulina	110,000	227,000,000	1,512,500	78,000	74	147,000	1:1544
Odobaenus rosmarus	110,000	256,000,000	722,000	82,000	62	111,000	1:2300
Otaria jubata	250,000	475,000,000	2,000,000	181,000	74	140,000	1:2000
Balaenoptera physalus	62,000	800,000,000	550,000	62,000	13	157,000	1:5095
Phocaena communis	200,000	175,000,000	1,350,000	184,000	29	36,100	1:4850
Delphinapterus leucas	150,000	735,000,000	794,000	98,000	28	137,000	1:5560
Hyperoodon rostratus	111,000	557,000,000	918,000	90,000	15	77,000	1:7200

as the rods, which would make the enormous total for the outer nuclear layer in Phoca barbata more than 4,000,000,000 nuclei. In all the species mentioned in table 1, the number of nuclei in the inner nuclear layer approached more closely that of the rods, being slightly less in most cases. Equally important is the comparatively small number of optic nerve fibers associated with this enormous number of retinal cells. Pütter estimated that in Balaenoptera physalus there were only 13 optic nerve fibers per square millimeter, giving a total of 157,000 for the entire nerve. In this case there would be about 5000 visual cells to a single optic nerve fiber. Considerable difference was found between the number of elements in embryonic and adult retinas. As a rule the nuclei were more abundant in the former.

Investigation into the numerical relations of the retinal cells in other mammals, though meagre and fragmentary, indicate a condition similar to that in man. Chiarini ('06) expressed in a general statement concerning the visual cells of the dog, the unsatisfactory condition in which we find this problem in mammals, when he said, "The cones are less numerous than the rods."

Franz ('09) has given a definite enumeration of the numerical relations of the retinal elements for the central and peripheral regions of the retina in birds. The nuclei were found in eight species to be more numerous at the center than at the periphery. In the fundus of the retina of Motacilla alba there were on a line 0.1 mm. in length 266 nuclei in the inner nuclear layer, 60 in the outer nuclear layer, and 50 in the ganglionic layer; but over the same distance at the periphery there were only 40 nuclei in the inner, 20 in the outer, and 4 in the ganglionic layer. To find the total number in one square millimeter, Franz multiplied these numbers by 10 and squared the product. On this basis the fundus was stated to have in Motacilla 250,000 ganglion cells and 360,000 rods and cones, in Bubo 36,000 ganglion cells and 78,400 rods and cones to a square millimeter.

I have been unable to find any enumerations of the retinal elements of reptiles. It is important to note, however, that the so-called rods are said to be wanting in some species. Thus Ramón y Cajal ('94) says, "In die Retina der Eidechse die Stäb-

chen fehlen." The number of elements involved is probably greater in eyes of equal size in reptiles than in amphibians, because of the larger size of the elements in the latter.

Detailed enumerations of retinal elements in amphibians have not been made, so far as I can learn, but Howard ('08) estimated that the rods, cones, and double-cones in the retinas of Necturus based on estimates in the fundus, were in the relation 4 : 1 : 1 respectively; but he made no mention of his method of obtaining this result. The outer nuclear layer is said by him to consist of a single layer of nuclei in the fundus and a double layer at the periphery, each rod and each cone is described as having a nucleus and each double-cone, two nuclei. Schultze ('67) believed that there was only one nucleus to each double-cone.

Franz ('05) has found the number of nuclei per square millimeter in the outer nuclear and ganglionic layers in the number of selachians. The nuclei, in most cases, were found to be more numerous about the center than at the periphery. In Acanthias blainvilli the number of nuclei in the outer nuclear layer near the center of the retina was 24,000 per square millimeter and in the ganglionic layer in the same region 1200; in Galeus galeus near the center there were 75,000 nuclei to a square millimeter in the outer nuclear layer and 1500 in the ganglionic layer. In the deep-sea selachians there was a great increase in the number of nuclei in the outer nuclear layer and a decrease in the ganglionic layer. Thus, in the fundus over an area of one square millimeter there were in the retina of Chimaera monstrosa 100,000 nuclei in the outer nuclear layer and only 600 in the same area in the ganglionic layer.

Enumerations of the visual elements in the eyes of invertebrates have been made in some cases. Parker ('90) placed the number of facets in an adult lobster's eye at 13,500 and ('95) the number of ommatidia in the eye of an adult crayfish (Astacus) at 2500. Hesse ('00) estimated that there were between 2100 and 2400 rods in the eye of Pecten jacobaeus. Among cephalopods, the retina of Loligo was said to contain 162,000 rods per square millimeter and of Scaeurgus only 26,000, with other species ranging between the two.

B. Optic nerve

Estimates of the number of optic nerve fibers, have been confined chiefly to the optic nerve of man. Kuhnt ('75) found 200 fibers in the diameter of the optic nerve of a new-born child, and 220 to 240 in that of a man forty years old, giving totals of approximately 31,400 and 40,000 respectively for the entire cross-sections. Krause ('76) put the number at 1,000,000 at least, but later ('80) reduced it to 400,000 including both large and small fibers, and an equal number of very small fibers. Salzer ('80) also worked on the optic nerve of man and from three nerves obtained an average of 437,745 fibers.

The only important contribution, to which I have had access, relating to the number of optic nerve fibers in amphibians is a short paper by Lauber ('02), who counted 450 fibers in a cross-section of the optic nerve of Cryptobranchus japonicus.

Among invertebrates Parker ('95) determined that seven fibers were connected with each ommatidium in the crayfish eye. This gave a total of 8085 retinal fibers in the case of a young crayfish and 16,625 in an old one. Proximal to the fourth optic ganglion these totals were reduced to 2021 fibers in the former and 4156 in the latter.

It is a generally credited theory that the majority of the optic nerve fibers originate in the retina and pass centripetally along the optic stalk to the brain, and that a smaller number arise in the brain and pass centrifugally to the retina. This view has the support of such investigators as His ('90), Ramón y Cajal ('91) and Robinson ('96). On the other hand Balfour ('81) did not hold this view, believing rather that the fibers of the optic nerve were derived from a differentiation of the epithelial cells of which the nerve was at first formed.

The axis-cylinders of the optic nerve in a majority of vertebrates are small and medullated. In some amphibians, viz., urodeles, the optic nerve was described by Osborn ('88) as 'greatly reduced,' and in some examples of Necturus there was stated to exist, in the adult condition a persistent lumen which opened into the *T*-like expansion of the third ventricle of the brain. Kings-

bury ('95), likewise, describes the optic nerve of Necturus as hollow for a portion of its length and states that its fibers are entirely amyelinic. This latter condition is said by Edinger ('92) to be the case in the young of certain other amphibians.

After a careful survey of all the literature at hand bearing on the numerical relations of the histological elements of the optic nerve and retina, I have failed to find for any vertebrate a consistent enumeration of these elements which has been carried out in such a way as to give reasonably safe grounds for comparisons. I have, therefore, undertaken this task in reference to Necturus.

III. MATERIAL AND TECHNIQUE

A. Material

It has long been known that the retinal elements in amphibians are very large as compared with those in other vertebrates. Howard ('08) gives the dimensions of the outer and inner segments of the rods in several species of amphibians as follows: In the outer segment the length varied from 24μ in Triton to 76μ in Bufo, and the width from 6μ in the frog to 12μ in both Triton and Salamandra. The outer segments of the rods in Necturus are said to be 36 to 40μ long and 12μ wide. Measurements made by myself on the diameter of the cones of Necturus just distal to the ellipsoids averaged 4.8μ, and through the ellipsoids, where visual cells first come in contact with neighboring visual cells, they averaged 10μ. The diameters of the rods through the ellipsoids was slightly larger than that of the cones. Slonaker ('97) states that "Amphibia have not only long rods, but the thickest found in vertebrates." In speaking of the cones, he says, they have the "greatest diameter in mammals." Table 2 shows the relative sizes in micra of the rods and cones in representatives from all classes of vertebrates. The length of the elements is non-essential for my purpose, but the diameter is of especial significance because it is one of the most important factors in determining the number of elements which can occupy a given space, when they are crowded together as are the visual cells in the vertebrate retina. From the measurements quoted

it will be seen at once that, because of the large diameter of the visual cells, the number of these elements in a given area will be fewer in the amphibia than in any other class of vertebrates. The two conditions, viz., large diameter and fewer elements, unite to make the amphibian retina especially favorable for a study of the numerical relations of the constituent parts. With this point in mind I have selected for my investigation the adult form of Necturus maculosus (Raf.), the common 'mud-puppy' of the fresh water streams and lakes of eastern Canada and of middle and southern United States (Cope, '89). The specimens were secured by the Zoölogical Department from Venice, Ohio.

TABLE 2

Comparative sizes of the outer segments of rods and cones in representatives of the different classes of vertebrates (after Müller '56)

SPECIES EXAMINED	DIMENSIONS IN MICRA OF THE OUTER SEGMENTS OF THE			
	Rods		Cones	
	Diameter	Length	Diameter	Length
Man	1.5 – 1.8	40.0 – 60.0	4.0 – 6.0	32.0 – 36.0
Pigeon	2.6 – 3.3	20.0 – 28.0	1.0 – 5.0	25.0 – 30.0
Chameleon			1.0 – 1.3	60.0 – 80.0
Frog	6.0 – 7.0	40.0 – 60.0	5.0	20.0 – 28.0
Perch	2.6	40.0 – 50.0		8.0 – 12.0

Upon their arrival at the laboratory they were transferred at once to a fresh-water cement aquarium in a dimly lighted part of the basement of the Museum of Comparative Zoölogy, where they were easily kept in a healthy condition.

B. Technique

I have found that the technique usually employed for the retina does not give satisfactory results when applied to the optic nerve; for this reason I have been obliged to make separate preparations for the two structures.

In order to secure material for a reliable enumeration of the retinal elements, a fixation fluid was necessary which would not wrinkle the retina, and at the same time could be followed by

successful double staining, to which I shall refer later, whereby small fragments of rods could be distinguished from bits of cones. The large size of all the retinal cells in Necturus has been a great aid in the identification, and in the accuracy of the counts, of the separate elements of the retina. The most successful results were obtained by fixation in Kleinenberg's picro-sulphuric mixture. My method was as follows: Live animals were placed in a bowl of tapwater in which a few small crystals of chloretone had been dissolved as a means of preventing the discharge of slime (Cole, '02). Chloroform was then added gradually until the animals were thoroughly anesthetized. The eyes were quickly removed and placed in picro-sulphuric acid, care being taken to free them from as much superfluous tissue as could be done quickly. To keep the orientation, a piece of skin was left attached to the dorsal side of the eyeball, differences in shape of the pieces serving to distinguish right and left eyes. I found the pieces of skin useful also in orientating the eyes in paraffin. The best results were obtained by immersing the unopened eyeballs in the picro-sulphuric mixture for four or five hours. They were then rinsed in distilled water a few minutes and dehydrated by passing them gradually through 35 per cent, 50 per cent, 90 per cent, and 100 per cent alcohol over a period of two days. When the eye was sufficiently hardened (90 per cent alcohol), the front face was cut away with a sharp razor, and the lens removed. Early in my work I found that the heat of a paraffin bath, extending over a time sufficient to insure saturation with paraffin, caused considerable shrinkage of the sclera and wrinkling of the retina. I, therefore, followed Bütschli's chloroform method of de-alcoholization. Transfer from the chloroform-paraffin mixture of this method to hard paraffin was completed by the evaporation of the chloroform over a water bath at about 60°C. and a five minute immersion in hard paraffin melting at about 56°C.

Sections 8μ thick were made in one set of eyes parallel to the antero-posterior[1] plane of the eye and passing through the optic

[1] The terms 'anterior,' 'posterior,' 'dorsal' and 'ventral' as applied to the eyeball in this account, are used in the sense of comparative anatomy; i.e.. 'anterior'

nerve, and in another set parallel to the dorso-ventral plane, and passing through the optic nerve. Series of sections 6μ thick were made through the entire thickness of the retina tangential to the surface of the eyeball in the anterior, posterior, dorsal, and ventral regions and in the fundus. The sections were stained in Heidenhain's iron haematoxylin as a base, and in a 70 per cent alcoholic solution of eosin as a counter stain. Thirty minutes in the mordant (2 per cent ferric alum) and one hour in the haematoxylin gave very satisfactory results. The excess of stain was washed out in ferric alum of the same strength as the mordant, and the washing was continued until all traces of the haematoxylin had disappeared from the outer segments of the rods and from the reticular layers. At this stage the nuclei of the outer and inner nuclear layers and of the ganglionic layer were clearly defined and light blue in color. The nuclei of Müller's fibers were stained a deep blue to blue-black and contrasted sharply with the lighter blue of the surrounding nuclei. Control was kept over the process of destaining by examining the slides every few seconds under the microscope. Two minutes in the counter-stain were sufficient to color the outer segments of the rods bright red. The outer and inner reticular layers appeared as broad red fibrous bands separating the inner nuclear layer from the outer nuclear and the ganglionic layers, respectively. The strands of Müller's fibers, which stretched radially outward from the internal limiting membrane, were stained an intense red. It is clear that the selective qualities of the stains employed rested primarily with the haematoxylin, for in the process of reducing the over-stain of the base the outer segments of the cones, the nuclei of Müller's fibers, and the ellipsoids, retained their color longer than the other elements, thus indicating their greater chemical affinity for the basic stain. Although the success which attended fixation in picro-sulphuric acid and double staining with haematoxylin and eosin made other methods superfluous, nevertheless some entirely successful preparations were

refers to that part of the eyeball which is nearest the anterior end of the animal ('internal' in human anatomy), 'dorsal' to that part which is nearest the dorsal midline ('superior' in human anatomy), etc. The deep part of the eyeball, often called the posterior part, is here referred to as the 'fundus.'

made with the use of other reagents. Both the vapor of 2 per cent osmic acid and vom Rath's picro-osmo-platinic-chloride-acetic mixture gave excellent preservation of the retinal elements, though the outer segments of the rods and cones were over-blackened. Good preservation and successful double-staining were obtained by fixation in either Perenyi's fluid, Fol's mixture, or 7 per cent nitric acid, followed as before with haematoxylin and eosin stains.

I have already called attention to the non-medullated character of the optic nerve fibers in Necturus, hence the usual fixation fluids in which osmic acid is used to stain the medullary sheaths are ineffective for the optic nerve fibers, in this animal. Vom Rath's fluid gave excellent preservation of the supporting and vascular tissues, but was unsatisfactory for the nerve fibers. Ranson's ('09) modification of Ramón y Cajal's silver-nitrate method for non-medullated nerve fibers was tried without success. With another modification of this method, that of Mullenix ('09), I succeeded in staining the fibers, but the definition was poor. The only method tried which brought out the fibers distinctly was a modified form of Bielschowsky's ('03) method. In order to secure the necessarily rapid fixation of the proximal portion of the optic nerve, I cut away the tissues surrounding the skull, which was then split at both anterior and posterior ends. This permitted the formalin to enter the brain cavity quickly. The non-medullated fibers when impregnated by this method appeared in longitudinal section as sharply defined somewhat undulatory, brownish-black to black lines. In cross-section (figs. 10, 11, 12) they were irregular black spots or streaks in a yellowish-brown matrix. Dehydration and de-alcoholization were carried out as with the retina. Cross-sections of the optic nerve 5μ thick were made close to the chiasma and as near as possible to the eyeball.

The results obtained with Bielschowsky's fluid have justified its use in this case. There is no doubt in my mind that the irregular black spots and streaks referred to above are nerve fibers. I was fortunate in having a longitudinal section of an optic nerve turn up slightly so that the fibers could be seen to end as small

black spots of different sizes. In a longitudinal section individual fibers could be traced only a short distance. At the distal end they spread out in bundles along the inner margin of the retina and disappeared radiating outward between the nuclei of the ganglionic layer. I was wholly unable to detect a union between the ganglion cells and the optic nerve fibers, and I am unable therefore to state the exact relation of the optic nerve fibers to the retinal elements.

IV. OBSERVATIONS

A. Measurements

a. Retinal layers. The eyeballs of Necturus lie well forward on the dorso-lateral aspect of the head, where they appear as slightly arched whitish bodies. They are unusually small for the size of the animal, and are approximately spherical in shape. The retina, which is closely applied to the sclera, has, therefore, the shape of a *zone of one base* whose area is only slightly less than that of a hemisphere. Since the area of a zone of one base is equal to the area of a circle whose radius is the chord of the generating arc, the area of the retina in Necturus is equal to the area of a circle whose radius is the shortest distance from the periphery of the retina to the center of its fundus. To obtain this center I made a camera drawing ($\times$ 101) of a median section of the eye, and erected a perpendicular at the middle point of the chord joining the ends of the retina at the ora. The point of intersection of the perpendicular and the retina marks the center required. The chord joining this center to the periphery is equal to the radius of a circle whose area is that of the zone of one base, which marks the limits of the retina. In many cases the center lay directly in the optic nerve, while in nearly all the remaining eyes, it lay very close to that nerve. By this method I have calculated the minimum, maximum, and average areas of zones of one base (which hereafter I shall refer to as *zones*) for 14 retinas, the three zones coinciding with the external limiting membrane, the middle part of the inner nuclear layer and the middle of the ganglionic layer (fig. 1, *cd, xz, vw*). The results

of my measurements are given in table 3. Eight retinas were measured in the antero-posterior and six in the dorso-ventral plane. Variations in the thickness of the retinas (figs. 2 to 4), measured from visual cells to internal limiting membrane, and irregularities of the retinas, especially at the periphery, are the causes of the differences of area in retinas nos. 3 and 8, 5 and 6, and 10 and 14.

In order to enumerate the retinal elements, it was necessary to adopt unit areas small enough to avoid the distortion at the margin of the microscopic field and large enough to include a great number of elements. Convenient sizes were found in a circular area of 0.013 sq. mm. for the visual cells and one of 0.0078 sq. mm. for the other elements. The areas of the retina in the zones at the different levels were duly considered, so that the number of visual cells and nuclei of the outer nuclear layer was

TABLE 3

Calculated areas of zones corresponding to the area of the retina in Necturus and passing through (1) the external limiting membrane, (2) the middle of the inner nuclear layer, and (3) the ganglionic layer

DESIGNATION OF EYE	PLANES OF SECTIONS	AREAS OF ZONES IN SQUARE MILLIMETERS AT THE		
		External limiting membrane	Middle of inner nuclear layer	Middle of the ganglionic layer
1	Antero-posterior..	12.5664	11.7094	10.9073
2	Antero-posterior..	13.4484	11.8309	10.3138
3	Antero-posterior..	15.1777*	13.5815	12.0693
4	Antero-posterior..	13.8940	12.2255	10.6535
5	Antero-posterior..	15.0399	13.6455	12.3176
6	Antero-posterior..	15.0399	13.8940	12.8165
7	Antero-posterior..	12.0693	10.9133	9.8120
8	Antero-posterior..	15.1777*	14.0721*	13.0690*
9	Dorso-ventral.....	14.6303	12.1947	9.9743
10	Dorso-ventral.....	10.8184†	9.7028†	8.6916†
11	Dorso-ventral.....	12.5664	11.4616	10.4257
12	Dorso-ventral.....	13.2541	11.8005	10.4257
13	Dorso-ventral.....	13.9602	12.3796	10.9073
14	Dorso-ventral.....	10.9351	9.7569	8.6916†
Average areas of zones in square millimeters....................		13.4698	12.0834	10.7910

* Maximum areas

† Minimum areas

based on an area obtained for the zones at the external limiting membrane; Müller's fibers and ganglion cells for the zone through the ganglion layer; and nuclei for the inner nuclear layer from the area of the zone passing through the middle of this layer. The number of unit areas in each zone was found by dividing the number of square millimeters in the zone by the number of square millimeters in the unit area. The total number of elements in a layer obviously equaled the product of the number of areas into the number of elements per unit area. The methods employed for the other layers were evidently unsuited for enumerations of the inner nuclear layer, for in this layer we have to do with solid content rather than surfaces. For this layer I have resorted to counting the nuclei in a cylinder with a head of 0.0078 sq. mm. and with its long axis placed radially. The length of the cylinders was determined by the number of microtome sections necessary to pass completely through the layer, the number of sections being eight in each of the two cases counted. The number of such cylinders was found by dividing the area of the zone of the inner nuclear layer by the area of the cylinder head. In all cases where elements touch the boundaries of the unit areas (figs. 5 to 9) they were rated at one-half their value within the field.

b. Cross-sections of the optic nerve. The areas of the cross-sections of the optic nerve varied according to their location (figs. 10 and 11). In all cases examined the nerve was very much smaller proximally than distally, in one instance the former amounting to little more than one-third the latter (table 15). My method of calculating the cross-sectional areas of the nerves was modelled after that employed by Parker ('95) on the optic nerve of Astacus. Camera drawings of all the sections to be measured were made on paper of uniform thickness and weight; these were then cut out and weighed. A standard unit 0.0015 sq. mm. of the same magnification was also cut out and weighed. I could then easily obtain the required areas from the arithmetical formula:

$$X \propto Y \text{ when } T \text{ is constant}$$

where X equals the area of the cross-section, Y equals the weight

of the drawing, and T equals the thickness of the paper. In sections near the chiasma allowance was necessarily made for the lumen of the stalk, which becomes obliterated distally.

B. Enumerations

a. Visual cells. The sections used in enumerating the visual cells were cut tangentially to the surface of the retina in the five regions already referred to, passing through the outer segments of the rods and cones, and through the paraboloids of the double-cones (fig. 1, *ab*). The visual cells, therefore, were seen in cross-section, and because of their structure, large diameter, and differential staining qualities they were easily distinguished from one another. I was unable to detect any plan in the arrangement of the rods and cones, such as Schwalbe ('87) has depicted, but noted a marked tendency for five or six elements of a kind to run in lines, a feature which I think is without real significance. The association of rods or cones in groups of six or eight. (figs. 6 and 8) was of frequent occurrence. Their usual distribution may be described as irregularly scattered, and free from contact along their outer segments with neighboring elements (figs. 5 to 9). With a magnification of 615 diameters the cross-sections of the rods were bright red, circular to oval in outline, and with the characteristic vacuolated structure described by Howard ('08). They were larger than the cones and somewhat more numerous. The cones appeared as small round, blue-black, homogeneous bodies scattered irregularly over the field; the double-cones were far less numerous and much larger than either the rods or the cones. As one should expect, the paraboloids were fused along one side (figs. 5 to 9), giving the appearance of double-elements. Their structure was reticular and took the stain only slightly. In addition to the elements already mentioned, there were a few whose identity could not be established with certainty, due, I think, to disintegration having set in before they were reached by the fixative fluids. They constituted only a very small percentage of the total number of visual cells.

Studies of the cross-sections of the cells in the five regions described, showing the close relation between the number of rods, cones, and double-cones, in the different positions, are

clearly set forth in table 4. I have taken the averages from five retinas in each case, right and left eyes being used indiscriminately. Twenty eyes were used, and in five cases counts from two different regions of the same eye were made, and in two cases right and left eyes from the same animal were considered. Averages per unit field for the right and left eyes respectively show a slightly greater number of rods and cones, and a smaller number of double-cones and doubtful elements in the left eye. These differences are shown in table 5. The maximum and minimum counts are, however, so close in every case as to lead me to believe that the number of elements involved in right and left sides is approximately the same, and I shall therefore make no discrimination between the two.

TABLE 4

Number of rods, cones, double-cones and uncertain elements in unit areas of 0.013 sq. mm.

DESIGNATION OF EYEBALLS	REGIONS OF THE EYEBALL IN WHICH THE UNIT AREAS WERE LOCATED																			
	Anterior				Posterior				Dorsal				Ventral				Fundus			
	*R**	*C*	*D*	*X*	*R*	*C*	*D*	*X*	*R*	*C*	*D*	*X*	*R*	*C*	*D*	*X*	*R*	*C*	*D*	*X*
7 *r*																	46.0	39.5	12	1
8 *l*	70.5	49.5	14	0													56.5	47.5	14	1
16 *r*					35.0	44.5	21	0												
21 *l*									42.5	41.0	14	2	52.5	46.5	14	4.0				
22 *l*	49.2	41.0	14	2																
23 *r*																	41.0	37	17	0
23 *l*													65.5	28.5	10	6.0	37.5	31	16	0
24 *r*																	50.0	26	20	2
24 *l*	66.0	42.5	25	0																
25 *l*													64.0	42.0	8	9.5				
26 *r*	47.5	47.0	14	0	59.0	37.5	19	1												
28 *r*									50.5	37.0	12	1								
31 *r*									43.0	42.5	15	4	70.5	43.0	5	4.0				
33 *l*													45.0	49.5	18	2.5				
38 *r*	56.0	29.5	12	0																
40 *l*					48.0	54.0	10	1												
56 *r*					43.5	38.0	14	0												
57 *r*					55.5	41.5	25	0												
59 *r*									42.5	36.5	10	0.5								
60 *l*									40.5	28.0	8	3								
Average of 5 retinas	57.9	41.9	15.8	0.4	48.2	43.1	17.8	0.4	43.6	41	11.8	1.9	59.5	41.9	11	5.2	46.2	36.2	15.8	0.8

**R*, rods; *C*, cones; *D*, double-cones; *X*, uncertain elements; *r*, right eye; *l*, left eye.

From tables 4 to 7 it will be seen that the rods are generally more numerous per unit field than the cones and double-cones. Exceptional cases exist, however, where the cones are found to be more numerous than the rods (table 4, nos. 16 *r*, 33 *l*, 40 *l*), and I am convinced after examination of a great number of retinas that such areas are not infrequent in every one of the five regions. The actual relative values of rods, cones, and double-cones and the total average number per unit fields, as well as the total average number per five unit field, are given in table 6, where the rods consistently outnumber the cones. In table 7 I have brought together many data for the visual cells. Between the maximum and minimum retinas there is a variation of 25 per cent, at least, in the total number of visual cells.

TABLE 5

Average number of retinal elements per unit area in right and left eyes

SIDE EXAMINED	UNIT AREA IN SQUARE MILLIMETERS	AVERAGE NUMBER OF			MAXIMUM NUMBER OF			MINIMUM NUMBER OF		
		Rods per unit field	Cones per unit field	Double-cones per unit field	Rods per unit field	Cones per unit field	Double-cones per unit field	Rods per unit field	Cones per unit field	Double-cones per unit field
Right	0.013	49.2	38.2	15	70.5	47	25	35	26	5
Left	0.013	53	41.7	12.7	70.5	49.5	25	37.5	28	8

TABLE 6

Average numbers of visual cells in the five selected unit areas (see table 4)

POSITIONS OF UNIT FIELDS	AVERAGE NUMBERS OF THE SEVERAL KINDS OF RETINAL ELEMENTS PER UNIT AREA IN THE FIVE REGIONS OF THE EYE				AVERAGE NUMBER OF ELEMENTS PER UNIT AREA
	Rods	Cones	Double-cones	Uncertain elements	
Anterior	57.9	41.9	15.8	0.4	116.0
Posterior	48.2	43.1	17.8	0.4	109.5
Dorsal	43.6	41.0	11.8	1.9	98.3
Ventral	59.5	41.9	11.0	5.2	117.6
Fundus	46.2	36.2	15.8	0.8	99.0
Total number of the several kinds of elements in five combined unit areas	255.4	204.1	72.2	8.7	540.4

To correlate the number of visual cells with the number of nuclei in the outer nuclear layer, it was necessary to count each double-cone as two units, on the assumption that each double-cone has two nuclei. This view is held by Howard ('08), who found two nuclei associated with each double-cone in macerated material. My own observations on sectioned material confirm this relation. The proportions of rods to cones and to double-cones approximates 3.5 : 2.8 : 1, respectively, which is a decided increase in the relative number of cones over that given by Howard.

b. Outer nuclear layer. The outer nuclear layer, when examined in radial sections of the eye (figs. 1 to 4) was seen to consist of a complete single external row of nuclei with here and there additional nuclei lying directly against its inner side. In calculating the number of nuclei in the 'external' sheet of which the 'single row' is the section, I have used the method already employed for the visual cells. The numbers of nuclei per unit area in the external sheet are given in table 8. In the fundus the numbers are slightly less than in the other regions. In order to include the partial 'internal' sheet in my enumerations, I counted the nuclei in the external and internal rows in median sections from periphery to periphery in eight retinas. In the

TABLE 7

Total numbers of visual cells in retinas of minimum, maximum, and average sizes. The enumerations are based on the following average numbers of elements in a unit field of 0.065 sq. mm. area: rods, 255.4; cones 204.1; double-cones, 72.2; uncertain elements 8.7; total visual cells, 540.4 (table 6)

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQUARE MILLIMETERS AT EXTERNAL LIMITING MEMBRANE (TABLE 3)	NUMBER OF UNIT FIELDS IN RETINAS	TOTAL NUMBER OF				
			Rods	Cones	Double-cones	Uncertain elements	Visual cells
Minimum..	10.8184	166.437	42,508	33,970	12,017	1,448	89,943
Maximum....	15.1777	233.503	59,637	47,658	16,859	2,032	126,185
Average of 14 retinas....	13.4698	207.228	52,926	42,295	14,962	1,803	111,986

external row there was an average of 422 nuclei, as compared with 121.1 in the internal row. Since the nuclei of the two layers have the same average diameters in the plane tangential to the surface of the eyeball, the ratio of the number of nuclei in the external layer to the number in the internal layer is 422 : 121. The total number of nuclei in each of these layers as well as in the whole outer nuclear layer is given in table 9. By comparing tables 7 and 9, the number of nuclei in the outer nuclear layer is seen to exceed the number of visual cells by about 10 per cent; or, if directly compared for total numbers of elements, there are 121,000 nuclei in the outer nuclear layer to 111,000 visual cells.

c. Ganglionic layer. The nuclei of the ganglionic layer, which in radial sections of the eye (figs. 1 to 4), are seen to be a single

TABLE 8

Number of nuclei of the outer nuclear layer per unit area of 0.0078 sq. mm.

DESIGNATION OF THE EYEBALLS	REGIONS OF THE EYEBALL IN WHICH THE FIELDS WERE LOCATED				
	Anterior	Posterior	Dorsal	Ventral	Fundus
7 *r**					55.5
8 *l*					54
16 *r*		53.5			
21 *r*	51.5				
21 *l*			59		
22 *l*	60	60			
23 *r*					49
23 *l*				51.5	
24 *r*					47
24 *l*					43
25 *r*	42	60			
25 *l*			61		
26 *r*	58.5	59			
28 *r*			53	42.5	
31 *r*				57	
32 *r*			60.5	51	
38 *r*	49				
39 *l*				71.5	
40 *r*			56		
40 *l*		62			
Averages of 5 retinas	52.2	58.9	57.9	54.7	49.7

**r*, right eye; *l*, left eye.

layer thick, are larger, more rounded and less closely grouped than are the nuclei of the other retinal layers. Sections from the five visual regions (table 10) in five retinas show as before a slight variation in the average number of nuclei per unit area, but this I believe is due to accidental conditions and is not at all consequential. Contrary to the condition found in the outer nuclear layer, the number of nuclei in the ganglionic layer is greater in the fundus than elsewhere. In table 11 the average number of nuclei per area of 0.39 sq. mm. is given as 111.1 and the total number of nuclei in a retina of average size is about 30,000.

Thus we see that in passing centripetally through the layers of the retina there is a gradual decrease in the number of elements composing each layer, except in the inner nuclear layer, so that according to the views usually expressed, visual impulses arising in a number of visual cells are transmitted to the central organ over a greatly reduced number of elements.

d. Müller's fibers. Müller's fibers were counted to best advantage in tangential sections through the inner reticular layer close to the ganglion-cell layer, where they appeared as intensely red spots in a net-work of small light-red fibers. They occurred in pairs and triplets as well as singly (figs. 1 to 4). I have assumed that each fiber represents a cell with a nucleus lying generally in the inner nuclear layer. Slight variations in the number of

TABLE 9

Total numbers of nuclei of the outer nuclear layer in retinas of minimum, maximum, and average sizes

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQUARE MILLIMETERS AT EXTERNAL LIMITING MEMBRANE (TABLE 3)	AREA IN SQUARE MILLIMETERS OF UNIT FIELD	NUMBER OF UNIT FIELDS IN RETINAS	AVERAGE NUMBER OF NUCLEI PER UNIT FIELD IN EXTERNAL SHEET	TOTAL NUMBER OF NUCLEI IN EXTERNAL SHEET	TOTAL NUMBER OF NUCLEI IN INTERNAL SHEET	TOTAL NUMBER OF NUCLEI IN OUTER NUCLEAR LAYER
Minimum....	10.8184	0.039	277.394	273.4	75,840	21,764	97,604
Maximum....	15.1777	0.039	389.172	273.4	106,400	30,558	136,958
Average of 14 retinas.....	13.4698	0.039	345.379	273.4	94,427	27,097	121,524

TABLE 10

Numbers of the nuclei of the ganglionic layer per unit area of 0.0078 sq. mm.

DESIGNATION OF THE EYEBALLS	REGIONS OF THE EYEBALL IN WHICH THE FIELDS WERE LOCATED				
	Anterior	Posterior	Dorsal	Ventral	Fundus
7 *r**					31.5
9 *r*					19.5
16 *r*		25.5			
21 *r*	14	16.5			
21 *l*			19		
22 *l*	23				
23 *r*		23			23
23 *l*				16	
24 *r*					27.5
24 *l*	16.5				33.5
26 *r*	18.5	26.5			
28 *r*			18.5		
30 *l*				13.5	
31 *r*				25	
31 *l*			20		
32 *r*			25	24	
33 *l*				20	
38 *r*	18				
40 *r*			25.5		
40 *l*		32.5			
Averages of 5 retinas	18.0	24.8	21.6	19.7	27.0

**r*, right eye; *l*, left eye.

TABLE 11

Total numbers of nuclei of the ganglionic layer in retinas of minimum, maximum, and average sizes

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQUARE MILLIMETERS AT MIDDLE OF GANGLIONIC LAYER (TABLE 3)	SIZE OF UNIT AREA IN SQUARE MILLIMETERS	NUMBER OF UNIT AREAS IN RETINA	AVERAGE NUMBER OF NUCLEI IN FIVE UNIT AREAS	TOTAL NUMBER OF NUCLEI OF GANGLIONIC LAYER
Minimum........	8.6916	0.039	222.861	111.1	24,760
Maximum........	13.0690	0.039	335.102	111.1	37,230
Averages of 14 retinas........	10.7910	0.039	276.723	111.1	30,744

fibers per unit area occurred, but not sufficiently great to call for more than passing notice. In table 12 I have given the average numbers of fibers per unit area and the average numbers for five retinas in each of the selected regions. The fibers were counted in unit areas, in some cases in right and left eyes of the same animal, and in others in two regions in the same eye; but I could detect no characteristic differences. In table 13 the average number of fibers in five unit areas is given as 97.5 and the total number in the whole retina is between 21,729 and 32,672.

e. Inner nuclear layer. The inner nuclear layer presents in Necturus a variable condition, which makes it, of all the retinal layers, the most difficult in which to count the nuclei. It con-

TABLE 12

Number of Müller's fibers per unit area of 0.0078 sq. mm.

DESIGNATION OF EYEBALLS	REGIONS OF THE EYEBALL IN WHICH THE UNIT AREAS WERE LOCATED				
	Anterior	Posterior	Dorsal	Ventral	Fundus
7 *r*					21
8 *r*			21		
16 *r*		24			
17 *l*				24	
21 *r*	18	13			
21 *l*			17		16
23 *r*		21			13
24 *r*					21
24 *l*	22.5				20
25 *l*	13				
26 *r*	22				
28 *r*			15		
30 *l*				16	
31 *r*				20.5	
31 *l*			19		
32 *r*				24	
33 *l*				21	
38 *r*	20				
22 *l*		19.5			
40 *r*			18		
40 *l*		27			
Averages of 5 retinas	19.1	20.9	18.0	21.3	18.2

r, right eye; *l*, left eye.

TABLE 13

Total numbers of Müller's fibers in retinas of minimum, maximum, and average sizes

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQUARE MILLIMETERS AT MIDDLE OF GANGLIONIC LAYER (TABLE 3)	SIZE OF UNIT AREA IN SQUARE MILLIMETER	NUMBER OF UNIT AREAS IN RETINA (TABLE 11)	AVERAGE NUMBER OF FIBERS IN FIVE UNIT AREAS	TOTAL NUMBER OF MÜLLER'S FIBERS
Minimum........	8.6916	0.039	222.861	97.5	21,729
Maximum.......	13.0690	0.039	335.102	97.5	32,672
Averages of 14 retinas	10.7910	0.039	276.723	97.5	26,980

sists in its usual form of three layers of nuclei (fig. 1), of which the middle one has fewer nuclei than either of the other two. In addition a large part of the nuclei of Müller's fibers lie within it (figs. 1 to 3). The number of layers may be as numerous as four (fig. 3), or even five, or they may be as few as two (fig. 2); in one case I found only a single layer in a part of one side of the retina. Such variation makes any estimate of the number of cells unsatisfactory. Since the most frequent condition is the three layered one, I have determined the number of nuclei in a zone of this kind. Figs. 1 to 4 show that there is little difference in the 'tangential' diameters of the nuclei of the outer and inner nuclear layers. With any increase or decrease in the average tangential diameter of the nuclei of one of these layers over that in the other, the number of nuclei in a given belt, such as I have used in the inner nuclear layer, would be found to vary and I could not then make the proportion as stated, but if the average tangential diameters of the nuclei of the two layers is the same the relations between the two would be constant and my results would hold true. Since this is the case, the total number of nuclei in the two layers respectively should be nearly proportional to the number of rows as seen in radial section, and the area of the zone. If, then, the number of nuclei in the outer nuclear layer is 121,000 and this number varies as the number of rows and the area of the zone, it varies as their product, and the number of nuclei in the typical inner nuclear layer in the ret-

ina of average size should be about 167,000. This is a variation from the average estimates of two retinas of less than 5 per cent, which is negligible when we consider that the numbers in minimum and maximum retinas vary nearly 45 per cent of the former.

Since Müller's fibers are regarded as merely supporting structures and not a part of the nervous mechanism of the retina, they have been excluded from the final count of the nuclei properly belonging to the inner nuclear layer. The data for the inner nuclear layer of minimum, maximum, and average sized retinas are found in table 14. The extreme lower limit for the number of nuclei in this layer is approximately 97,000.

TABLE 14

Total numbers of nuclei of the inner nuclear layer in retinas of minimum, maximum, and average sizes

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQ. MM. AT MIDDLE OF INNER NUCLEAR LAYER, (TABLE 3)	CALCULATED NUMBER OF CYLINDERS IN RETINA	NUMBER OF NUCLEI PER CYLINDER			NUMBER OF NUCLEI		TOTAL NUMBER OF NUCLEI IN INNER NUCLEAR LAYER
			Retina 1	Retina 2	Average	In inner nuclear layer exclusive of Müller's fibers	Of Müller's fibers, (table 13)	
Minimum	9.7028	1243.948	121	142	131	141,228	21,729	162,957
Maximum	14.0721	1804.115	121	142	131	203,667	32,672	236,339
Averages of 14 retinas	12.8034	1549.153	121	142	131	175,959	26,980	202,937

f. Optic nerve fibers. As I have already said, the optic nerve was transected in two planes, viz., near its two ends, and the difference in the cross-sectional areas in these two regions was clearly established. When the fibers in the two regions were studied, those in the proximal portion seemed smaller and slightly more numerous to a unit area than in the distal portion. This led me to suppose that the total number of fibers was less proximally than distally, and my opinion was borne out by actual counts in the two regions in two optic nerves. Examination of other optic nerves indicated that similar numerical relations of the fibers existed in the two regions and was a characteristic feature of the optic nerve of Necturus. All counts were made on cross-sections of the optic nerves. Outline camera drawings ($\times$ 755) were made of cross-sections together with the nuclei

of the supporting tissue, all the largest fibers, and all the prominent markings of the optic nerve carefully sketched. The drawing was then marked off with coördinating lines and the rest of the optic nerve fibers were drawn in place and counted. By practice in counting I was able to make out and record the fibers in the sections with accuracy and speed.

I have counted the fibers in three of the nerves given in table 15 twice each. The results of the second counts were in each case so close to the first counts that I did not think it necessary to continue the process for every nerve; thus, in nerve no. 1 (fig. 12) I counted 2001 fibers on the first trial and 2003 on the second; in nerve no. 2, 1657 fibers on the first count and 1661 on the second;

TABLE 15

Areas of cross-sections of optic nerves and numbers of nerve fibers counted at different planes of transection

DESIGNATION OF NERVES	CALCULATED AREA IN SQUARE MILLIMETERS OF CROSS-SECTIONS OF THE OPTIC NERVE		TOTAL NUMBER OF FIBERS IN CROSS-SECTIONS	
	Close to eye	Close to chiasma	Close to eye	Close to chiasma
1	0.017		2002	
2	0.019		1659	
3	0.019	0.008	1780	853
4	0.026	0.009	1858	1071
5	0.027		2613	

and in nerve no. 5, 2616 fibers on the first count and 2611 on the second. The number of fibers appearing in table 15 for these optic nerves are averages obtained from the two counts. The complete results of my enumerations, together with the areas of the cross-sections of the optic nerve, are given in table 15. The average area near the eye was 0.0216 sq. mm. and contained by actual count an average of 1982.4 fibers or about 92,000 per square millimeter; the average of the cross-sections near the chiasma was 0.0085 sq. mm. with an average of 962 fibers, or approximately 113,000 per square millimeter. Although the proximal portion of the optic nerve is smaller in diameter than the distal part, it is, on the other hand, seen to be richer in fibers per unit area.

V. DISCUSSION

My intention in this investigation has been, as far as possible, to make a consistent enumeration of all the nervous elements in the retina and optic nerve in a single species of animal, and I have believed the logical choice of species to be some animal whose retinal cells are very large and few in number. Such an animal was naturally sought among the amphibians, and especially among those species whose eyes and optic nerves have undergone reduction in size, probably through diminished functioning powers, and whose histological elements were well known to be of unusual size. In Necturus I have found an animal in which both eyeballs and optic nerves are reduced in size; the retinal elements are extremely large, if not the largest known; and the optic nerve fibers are entirely non-medullated.

The nearly spherical shape of the eyeball in Necturus and the close application of the retina to the sclera have made the measurements of the retina comparatively easy and have led, I believe, to accurate results. The method employed has been described on page 417. The individual retinal cells are so large and free from contact with one another that I have been able to count and number the individual cells in each unit area. Following this the application of the unit area to the area of the retina has been a matter of simple mathematical calculation.

An attempt to estimate the total number of visual cells in the retina of Necturus by counting the rods, cones, and double-cones in a median section of the eye extending from periphery to periphery gave a total of 143,000 visual cells for an average sized retina. This number exceeds that found by the method finally adopted by about 31,000 cells or 28 per cent. Since in the latter method every element per unit area was counted and averages obtained as already stated, and the number of areas in a given retina is not subject to variation, I am convinced that the number of retinal cells in Necturus is close to the number obtained by this means, and that the method of counting elements in a line as a basis of enumeration of the visual cells should be rejected on the ground that it gives too large a number of cells.

Where the retinal cells are very small and numerous the difficulties of obtaining accurate counts are greatly increased. Hess ('05) has demonstrated in the retinas of Eledone and Sepia the great variation which exists in the number of visual cells in closely approximated regions in the same retina, and thereby implies the impossibility of obtaining reliable estimates of the total number of visual cells in a retina from the number in a given 'belt.'

It is commonly believed that the retinal cells in vertebrates are not evenly distributed over the retina. Franz ('05, '09) states that the visual cells are more numerous about the fundus than near the periphery; Howard ('08) calls attention to the double layer of nuclei near the periphery in the outer nuclear layer in Necturus; and my own observations on the same species show a slight variation in the numbers of the different elements in the different regions. Consequently I believe that estimates based on counts in a restricted region, as for example, the fundus, or the periphery, give no fair idea of the number of elements in the retina as a whole, and my observations show that estimates based on counts of elements in a line of definite length exaggerate the number of cells in a given retina. Nor can I accept Pütter's ('02) method, viz., that of computing from the diameter of a rod the number of visual cells that may be found in a square millimeter and in the retina as a whole. Figs. 4 to 9 show that in Necturus the interstices between the visual cells make up a large portion of the area of a zone passing through the outer segment of the visual cells, and these spaces must be considered in the retinas of all species of vertebrates. Without consideration of these spaces the number of elements obtained would greatly exceed the actual number present.

In order to avoid, then, what appear to me to be errors of method, I have taken great care to secure accurate counts per unit area in a number of regions of the retina in several animals. The retina of Necturus lends itself to a count of this kind, because the visual cells, the external sheet of the outer nuclear layer, Müller's fibers, and the ganglion cells are each represented by a single layer of nuclei.

The fovea, when present in the amphibian retina, is small. I have not been able to find it in Necturus, and Howard ('08) makes no mention of such a 'spot,' but Hulke ('67) and Chievitz ('91)[2] have seen it in Triton and Salamandra. The frequent grouping of 6 to 8 cone cells (figs. 5 to 9) in different parts of the retina of Necturus may, perhaps, compensate for the absence of a definite fovea.

Enumerations of nuclei in the outer nuclear layer (tables 8 and 9) bring out several important features. On the ground that each visual cell has its own nucleus there should be in the outer nuclear layer a number of nuclei equal to the number of rods and cones together, unless elements of another kind should be found in either or both the layers compared. The nuclei of the outer nuclear layer were found to outnumber the rods and cones by about 10,000. Undoubtedly a majority of the nuclei in the internal layer together with all those in the external layer should be associated with the visual cells; but there is a characteristic group in the internal layer, having their long axes at right angles to the long axes of the nuclei of the visual cells, which appear to me to have a different function. They may, perhaps, be horizontal cells, which Ramón y Cajal ('94) states are represented in amphibians by large and small nuclei at two different levels and constitute the outermost part of the inner nuclear layer. There are, too, in the internal layer a small number of Müller's-fiber nuclei (fig. 4) whose identity is clearly established by their heavy stain and fibers. A fourth kind of nucleus may occasionally wander into this layer. Fig. 2 shows how the outer limits of the inner nuclear layer may be thrown out of line and individual nuclei be protruded far into the outer reticular layer. This would seem to favor Bernard's ('00) contention that the nuclei of the retinal layers are constantly migrating outward to aid in the formation of new elements in the more outwardly lying layers. I have been able to observe an apparent migration of this kind only in the case cited.

Conditions in the inner nuclear layer show that it is the most variable of all the layers in respect to the number of its elements.

[2] See Slonaker, 1897.

I have called attention to the three-layer condition of its nuclei as the most characteristic condition, but two or four layers were not uncommon. Ramón y Cajal ('94) points out that the inner nuclear layer of amphibians consists of a number of 'types' of both amacrine and bi-polar cells, but I have made no attempt in this study to distinguish between them, and have considered the layer merely as a whole. Though the results obtained cannot be adapted to every retina because of the great individual variation, I am convinced that they are as accurate as possible for the three-layered average sized zone.

The ganglion-cell layer presents few features of special note in connection with the number of its nuclei. It consists uniformly of a single layer of loosely associated, large nuclei. A consideration of vital importance is the failure of Bielschowsky's impregnation method to demonstrate a union between the ganglion cells and the optic nerve fibers. Just what the real significance of this is, I am unable to say, and, when considered in relation to the number of optic nerve fibers in proximal and distal parts of the optic nerve, the interpretation becomes even more difficult.

Enumerations of the cross-sections of the strands of Müller's fibers per unit area in the inner reticular layer gave better results than enumeration of their nuclei per unit area in the inner nuclear layer, because at the plane of sectioning in the reticular layer there is no other structure with which they might be confused; secondly, the fibers stand out clearly in cross-section, and each fiber may be supposed with reasonable certainty to represent a complete unit; and, lastly, the chance of missing even a single fiber in the unit areas, was very small.

The objections to using the nuclei as a means of enumeration lie in the difficulty of distinguishing the nuclei of Müller's fibers from other nuclei in the layer; and secondly, in the fact that the nuclei of Müller's fibers are not always found within the limits of inner nuclear layer (fig. 4), in which case it would be impossible to count with certainty all the cells in a given field.

Enumerations of the optic nerve fibers were more tedious than difficult. The black ends of the fibers contrasted sharply with

the surrounding tissues. Over-blackened margins and the apparent fusion of occasional fibers did not prove to be insurmountable difficulties, for by focusing, the separate fibers could usually be distinguished, so that I believe the number of fibers counted in a cross section is very close to the actual number present.

In addition to variations in diameter in a given nerve, there are also individual variations which are considerable. Measurements near the chiasma are fairly uniform, but at the distal end of the nerve I found a range in area of 62.9 per cent. The number of fibers here correlates in some degree with the cross-sectional areas of the nerves. Thus in table 15, nerve no. 2, with a cross-sectional area of 0.019 sq. mm. has an average of 1659 fibers, and nerve no. 5, with an area of 0.027 sq. mm., has an average of 2613 fibers.

The number of fibers in other nerves than the optic nerve of Necturus have been counted or estimated in some instances. From Salzer's ('80) calculations there are between 60,000 and 70,000 fibers per square millimeter in the human optic nerve; Birge ('82) has counted 3550 fibers per square millimeter in the 7th spinal nerve of the frog, and 14,133 in the 10th; Hatai ('03) found a variation in the number of fibers in the spinal nerves of the white rat according to the position of the plane of transection, there being more in the proximal than in the distal planes, which is the reverse of the condition in the optic nerve of Necturus; Donaldson and Bolton ('91) record an average of 11,900 fibers to every square millimeter in the dorsal roots of the spinal nerves of man; Dunn ('00) has found that the total number of fibers innervating the hind foot of the frog is between 5000 and 6000; and on page 430 I have shown that in the optic nerve of Necturus there are about 100,000 fibers per square millimeter.

Thus we see that, although in general the visual apparatus of Necturus is described as degenerate, and the animal's habits have apparently brought about a reduced functional activity of the visual cells, the optic nerve appears to have more nerve fibers per unit area than any other nerve so far studied.

VI. THEORETICAL CONSIDERATIONS

Theoretical consideration of the optic functions is not the primary object of my investigation, and I shall enter into it only so far as my research indicates exceptional conditions in Necturus.

I have found in the visual cells that rods and cones alike develop at the extreme margin of the retina, the initial element being indifferently a rod or a cone. Since this is so, whatever differences there may be in the light perceiving potentialities between central and peripheral regions, they must depend largely on the degree of the functional development of the visual cells in these regions. The presence of rods and cones alike over the whole retina—if the theory that rods are organs for light and shadow perception and cones for color preception is true—indicates power to distinguish colored lights as well as light and shadow at the periphery as well as in the fundus. In this connection Reese ('06) found that Necturus responded to both red and blue lights, but since no analysis of the lights for purity of color or intensity was made, it is not evident to what the reactions were due. Pearse ('10) has shown that Necturus reacts readily to ordinary light stimuli through both the eye and the skin. From these investigations it appears that Necturus is capable of distinguishing colored as well as white light, but on this point there is need of further investigation.

I know of no positive evidence that horizontal cells exist in the retina of Necturus, but I know of no other explanation for the group of cells referred to on page 423 (figs. 2 and 3, *n*). The arrangement of the nuclei in the inner nuclear layer seems to me to preclude the existence of such cells in that layer. Since I have already shown that the nuclei of Müller's fibers may migrate or be pushed outward in some way from the inner nuclear layer into the outer nuclear layer, it is possible that a similar change in position has taken place with the horizontal cells, and that in time they have become permanent constituents of the outer nuclear layer. Investigation into the identity of these cells by Golgi's, or some other stain equally valuable for nerve processes, is very desirable.

Because of the great variability of the inner nuclear layer, I cannot help wondering what the influence of such variation may be on the sight of the animals. Can animals with five layers of cells in this region see better than those with two? Or does the number of cells involved have no effect whatever on the clearness of vision?

Enumerations of the optic nerve fibers in cross-sections of the optic nerve show that distally there are nearly double the number of fibers that there are in the proximal portion; a condition which suggests at once a dichotomous division of the axis-cylinders. Such an interpretation of the increase of the fibers distally would mean that a majority of the optic nerve fibers in Necturus have their origin in the brain, which is the reciprocal of the condition found by His ('90), Assheton ('92), and Robinson ('96) in other vertebrates. But Robinson also states that the optic nerve fibers arise in the retina and are more numerous near the retina even when the fibers have come to occupy the entire length of the optic stalk. The implication is that some of the fibers fail at this stage to reach the central organ.

In my treatment of the optic nerve of Necturus with Bielschowsky's fluid, I have been unable to demonstrate a morphological connection between the ganglion cells and the optic nerve fibers, but the fibers seem to pass between the nuclei of the ganglion cells and enter the inner reticular layer. If the fibers can in any way be shown to be joined to the ganglion cells, then we should have in the adult Necturus a condition similar to that found by Robinson in the embryos of higher vertebrates.

Since a direct union, then, between the optic nerve fibers and the ganglion cells is not an established fact, the origin of the fibers is a matter of speculation. Do the fibers originate wholly in the brain and pass centrifugally to the retina, branching within the optic nerve and ending freely in the retina? Or do they have their origin chiefly in the retina, in spite of the facts that staining with Bielschowsky's fluid fails to show positive connection between the ganglion cells and the fibers, and that only a half of them reach their destination in the brain? In consideration of these questions the influence of the degeneracy of the

eye, and the fallibility of the stain must be given due weight. I am unwilling to make any statement as to the origin of the optic nerve fibers in Necturus, and await with interest further investigation of this subject by other methods.

VII. SUMMARY

1. The proportion of rods to cones is about the same in all regions of the retina. Double-cones are wanting at the extreme periphery.

2. There is no particular plan of arrangement of the visual cells.

3. In the retina of average size there are about 110,000 visual cells, of which 53,000 are rods, 42,000 cones, and 15,000 double-cones.

4. The total number of visual cells varies with the size of the retina. In maximum sized retinas the number of visual cells is about 126,000; in minimum sized retinas, about 90,000.

5. The number of visual cells is less than the number of nuclei in the outer nuclear layer.

6. The total number of nuclei in the outer nuclear layer in an average-sized retina is 121,000. The number varies from 137,000 in maximum sized retinas to 97,000 in mimimum sized retinas.

7. The outer nuclear layer consists of two sheets; an external complete, and an internal loosely scattered sheet, with 94,000 and 27,000 nuclei, respectively, in the average-sized retinas.

8. The nuclei of the internal sheet of the outer nuclear layer consists of nuclei of the visual cells, of Müller's fibers, and possibly of horizontal cells.

9. The inner nuclear layer is the most variable of all the layers in the number of its elements. In an average sized retina there are approximately 176,000 nuclei in a layer composed of three sub-layers. The range in the number of nuclei in maximum and minimum retinas of this structure is 204,000 to 45,000.

10. The inner nuclear layer may have as many as five sub-layers or as few as two.

11. Nuclei of Müller's fibers lie chiefly in the inner nuclear layer, but a small number may be found in the outer nuclear layer. In an average sized retina there are about 26,734 of these nuclei, the maximum and minimum numbers being 33,000 and 22,000, respectively.

12. The number of ganglion cells in the smallest retina measured, was 24,758; in the largest, 37,229; and in an average-sized retina 30,464.

13. The optic nerve of Necturus varies in diameter with the plane of transection. Near the chiasma its cross section has on the average an area of 0.0085 sq. mm., and an average of 962 nerve fibers; near the eyeball the average area of the cross section is 0.0216 sq. mm., with an average of 1982 nerve fibers.

14. The proportion of histological elements of the retina and optic nerve are approximately as follows: visual cells 111, nuclei in the outer nuclear layer 121, nuclei in the inner nuclear layer, exclusive of Müller's fibers, 175, ganglion-cell nuclei 30, Müller's fibers 26, optic nerve fibers distally 2, optic nerve fibers proximally 1.

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EXPLANATION OF FIGURES

The drawings are all from Necturus maculosus (Raf.) All the outlines in figs. 1 to 12 were made with the aid of the camera lucida. The sections of the retina were stained in Heidenhain's iron haematoxylin followed by a 70 per cent alcoholic solution of eosin. Radial sections of the visual cells (figs. 1 to 4) were cut 8μ thick, and cross-sections (figs. 5 to 9) 6μ thick.

The optic-nerve fibers were stained in Bielschowsky's fluid in every case, and all sections were 5μ thick.

Fig. 1 Semi-diagrammatic drawing of a radial section of a normal retina of Necturus; *cd, xz, vw* designate three zones in which the areas of the retinas were obtained; *ab, mn, xz* and *vw* designate the 'levels' in which average counts, per unit areas, were obtained for the different layers of the retina. × 331.

Fig. 2 Semi-diagrammatic drawing of a radial section of a retina with two sublayers or 'sheets' in the inner nuclear layer. *n*, horizontal cell. × 331.

Fig. 3 Semi-diagrammatic drawing of a radial section of a retina with four to five sublayers in the inner nuclear layer. *n*, horizontal cell. × 331.

Fig. 4 Semi-diagrammatic drawing of a radial section of a retina with two nuclei, *m*, of Müller's fibers, in the outer nuclear layer. × 331.

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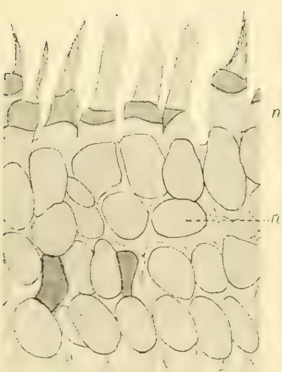

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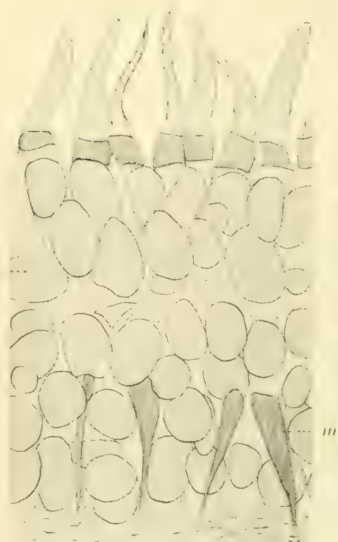

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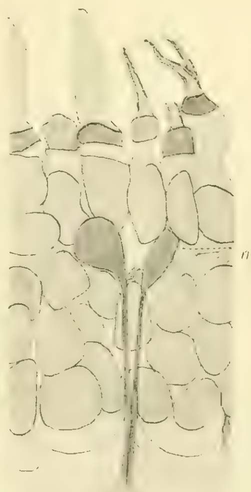

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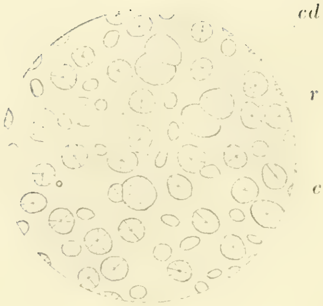

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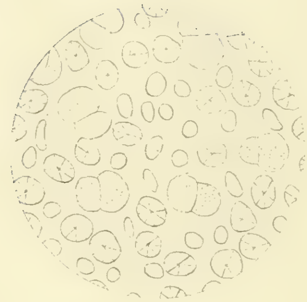

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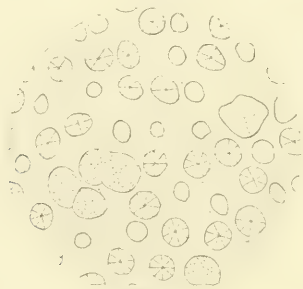

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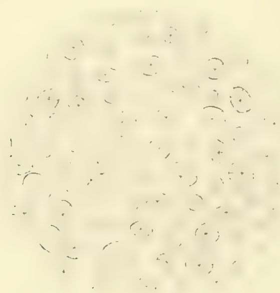

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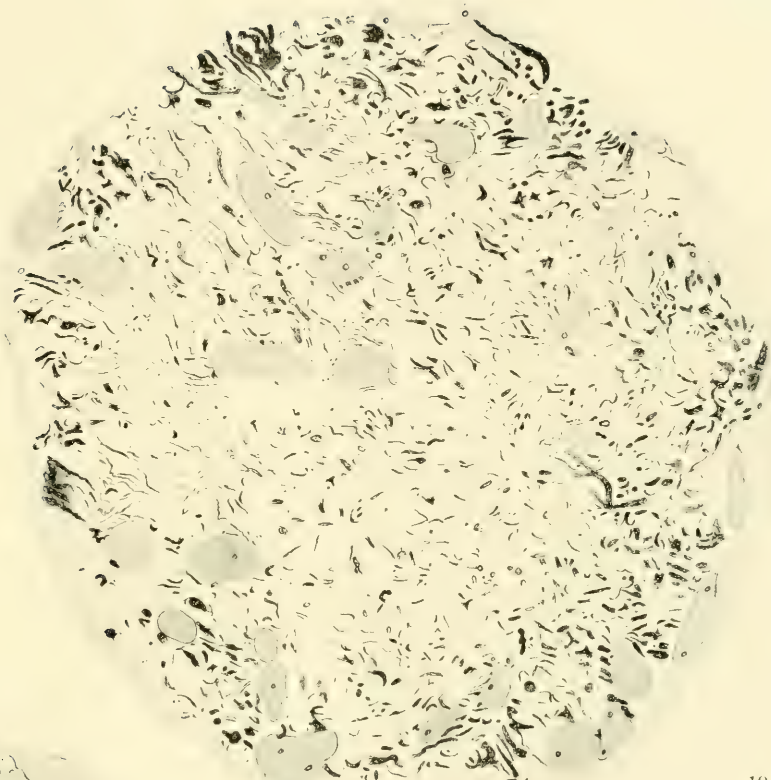
Figs. 5 to 9 Semi-diagrammatic drawings of cross-sections of the visual cells in five regions of the retina. Fig. 5, anterior; fig 6, posterior; fig. 7, fundus; fig. 8, dorsal; fig. 9, ventral; *r*, rod; *c*, cone; *cd*, double-cone. × 232.

Fig. 10 Semi-diagrammatic drawing of a cross-section of the optic nerve (no. 4, table 15) near the eyeball. × 431.

Fig. 11 Semi-diagrammatic drawing of the same optic nerve near the chiasma. *o*, lumen of the nerve. × 431.

Fig. 12 Semi-diagrammatic drawing of a cross-section of nerve (no. 1, table 15) near the eyeball. × 485.



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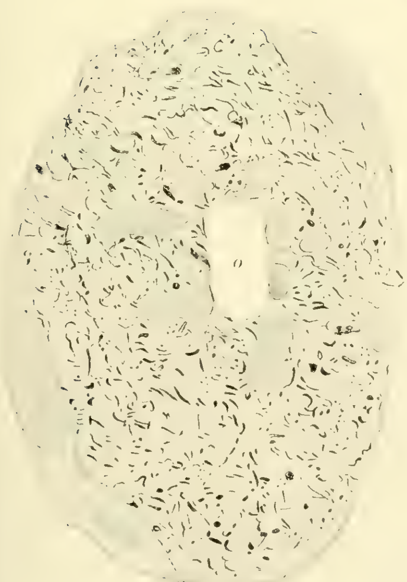

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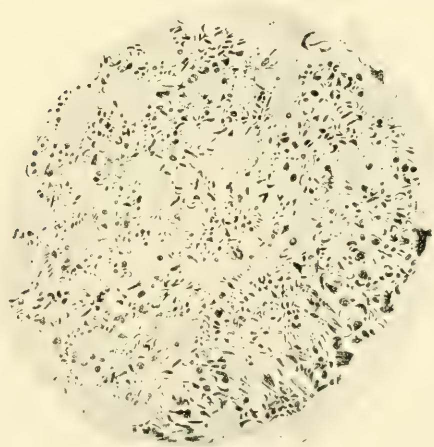

12

ON THE HISTOLOGY OF THE CRANIAL AUTONOMIC GANGLIA OF THE SHEEP

F. W. CARPENTER

From the Zoological Laboratory, University of Illinois, under the direction of Henry B. Ward, No. 19

TEN FIGURES

Both anatomical and experimental methods of investigation have established the fact that the autonomic nervous system of vertebrates consists, on its efferent side, of two sets of neurones. The first of these, the preganglionic neurones, have their cell-bodies in the central nervous axis, and send their neurites outward by way of the ventral roots of cerebro-spinal nerves to the autonomic ganglia. In the trunk region these preganglionic fibers pass from the spinal nerves in the white rami communicantes to the ganglia of the sympathetic trunks, or to ganglia more distally placed. In the cranial region, preganglionic fibers leaving the brain by the third, seventh and ninth nerves end in the ciliary, sphenopalatine, otic, submaxillary and sublingual ganglia, which constitute the autonomic ganglia of the head.

The efferent nervous impulses which thus reach outlying ganglia of the body are carried onward to peripheral tissues (involuntary muscle, glands) by the postganglionic neurones. These are multipolar nervous elements, the cell-bodies of which lie in the autonomic ganglia. Their distally directed neurites are in mammals, as a rule, nearly or quite devoid of myelin.

By employing modern methods of neurological technique, such as those introduced by Golgi, Ehrlich and Cajal, it has been possible to demonstrate very satisfactorily the nature of the endings of the preganglionic fibers in the autonomic ganglia of the trunk region. These ganglia, according to the terminology proposed by Langley, belong to the sympathetic and sacral subdivi-

sions of the autonomic system. The histological investigations of Aronson, Retzius, Dogiel, Huber and others on various vertebrates have led to the general conception of such endings as pericellular, intracapsular networks of fine fibrils, surrounding and in contact with the cell-bodies of the postganglionic neurones. These end nets are not to be confused with the intercellular fibers, often intertwined and concentrically arranged about the cell-bodies of the ganglia, but situated outside the cell capsules. Such fibers include the long extracapsular dendrites of the sympathetic cells, their non-medullated neurites, and the collaterals and terminal portions of the preganglionic fibers before the latter penetrate the cell capsules to give rise to the subcapsular nets.

Our knowledge regarding the terminations of preganglionic fibers in the cranial autonomic ganglia is, with the exception of the ciliary, less complete. In the ciliary ganglion pericellular nets under the capsules have been observed by a number of investigators. Michel ('94) and Kölliker ('94) were the first to call attention to their presence following the study of Golgi preparations of mammalian ganglia. Recently Sala ('10) and v. Lenhossék ('10), working with the Cajal silver nitrate method, have been able to secure excellent demonstrations of these end nets in the ciliary ganglia of man. That they are present also in birds has been shown by v. Lenhossék ('10, '11) and the writer (Carpenter '11), both of whom found, however, that in this group another form of termination of the preganglionic fibers occurs in the ciliary ganglion. This is the "calyx" ending and its modifications. Endings of this type have been seen also in the ciliary ganglion of reptiles by v. Lenhossék ('11a).

The histology of the remaining autonomic ganglia in the head region has received comparatively little attention from investigators.

Original descriptions of the cellular elements of the sphenopalatine ganglion are to be found, as far as I am aware, in three papers only, and in but one of these are the terminations of preganglionic fibers mentioned. The observations of Retzius ('80) on teased preparations of the ganglion taken from the sheep and cat furnished for a long time the only source of information regarding

its minute structure. Retzius found the cells mostly multipolar, with some bipolar elements in the cat. His method did not permit the study of nerve terminations in the ganglion. In 1894 v. Lenhossék described Golgi preparations of the sphenopalatine ganglion of the mouse, and gave us the only account of fibrillar end baskets around the ganglion cells. In the recent paper of Müller and Dahl ('10) a good description is given of the morphology of the cells of this ganglion in the horse, sheep, and man, but no mention is made of intracapsular end nets. The method of Bielschowsky was used. In one preparation (human) a pericapsular 'Nervenfasernetz' was observed by them, from which terminal fibers appeared to run to the cell-body. This will be referred to later.

In the otic ganglion the terminations of preganglionic neurones have not been demonstrated. The literature dealing with the histology of this ganglion is, in fact, very meager. Retzius ('80) ascertained from teased preparations that the ganglion contained multipolar cells in the rabbit, cat, sheep and man. Müller and Dahl ('10) have confirmed this, by means of the Bielschowsky method, for the horse, sheep and man.

The cells of the closely related submaxillary and sublingual ganglia have been described as multipolar elements by Retzius ('80), Huber ('96) and Müller and Dahl ('10). Huber alone saw pericellular end nets. These were observed in Golgi preparations of the ganglia from young dogs.

METHODS

The nerve terminations described in this paper were demonstrated by means of intra-vitam staining with methylene blue. The sheep's heads were brought to the laboratory about an hour after the animals had been killed, and injected through the carotid arteries with a 1 per cent solution of methylene blue in distilled water. The blood vessels were washed out before and after the staining by injections of Ringer's solution. Both this and the methylene blue solution were used at approximately body temperature.

After the ganglia desired for study had been dissected out, they were fixed over night in a 10 per cent solution of ammonium molybdate, then washed in running water, dehydrated in grades of alcohol, cleared in xylol, and embedded in paraffin. The sections were cut from 25 to 50 micra in thickness.

For comparison, preparations of the otic ganglion were made by the silver nitrate method of Ramón y Cajal. These were of some value in showing the form of the ganglion cells and their processes, but in them the terminal end nets were not differentiated.

OBSERVATIONS

In this investigation attention has been directed chiefly to the endings of the preganglionic fibers on the cell-bodies of the postganglionic neurones. In using methylene blue intra-vitam staining to demonstrate nerve terminations it has been found that the treatment of the ganglia in such a manner as to differentiate these clearly usually leaves the cell-bodies, about which the endings are arranged, partially or wholly unstained. On the other hand, when the cell bodies and their processes are deeply colored, the endings of the preganglionic fibers are almost always invisible. It follows, therefore, that the majority of my preparations are not suitable for the study of the morphology of the postganglionic neurones. There occur, however, here and there in the sections, ganglion cells sufficiently well stained to warrant giving some account of their structure.

The postganglionic neurones

In the sphenopalatine, otic and submaxillary ganglia of the sheep the cells are multipolar in character, with long, slender, sometimes branching dendrites, which penetrate the cell capsule, and often run for surprising distances among the intercellular fibers. Such cells are shown in figures 1, 2 and 3. The boundaries of the cell capsules are marked by dotted lines, except in figure 2, where the position of the capsule is indicated by several of its nuclei.

Among the processes given off by the cell-bodies, I have often found it difficult to distinguish the neurite from the dendrites.

In figure 1 the fiber marked *A* appears to be the neurite, in this instance arising from a dendrite. It may be traced through the thick section in which it occurs for the distance of nearly one-half a millimeter from its origin. It joins a bundle of fibers lying along the surface of the ganglion. Throughout its length, as far as it can be followed, it remains non-medullated.

Preparations of the otic ganglion stained by the Cajal silver nitrate method also show that the cells of this ganglion are multipolar; and, moreover, that some of them are fenestrated, resembling in this respect certain ciliary ganglion cells of mammals and birds (Sala, v. Lenhossék) and certain spinal ganglion cells of mammals (Cajal).

The ganglion which has proved most refractory in revealing the character of its cells is the ciliary. Here no cells with long, slender processes, such as those of the other ganglia, have been brought to light by methylene blue in the six ganglia examined by this method. In most cases no processes at all are apparent, but occasionally a cell showing a single thick, branching, extracapsular dendrite (fig. 4) has been observed. Whether such cells are numerous in the sheep's ciliary ganglion, or occur sporadically only, I cannot say. It would be unsafe to make the latter deduction merely because a partial and capricious stain like methylene blue shows them in comparatively few numbers.

Schwalbe ('79, '79 a) described the ciliary ganglion cells of the sheep, studied by the isolation method, as unipolar. In other mammals (cat, dog, monkey) they have been shown to be multipolar by Sala ('10) and Marinesco, Parhon and Goldstein ('08), who employed the method of Cajal. In some instances the dendrites extend beyond the cell capsules, in others they are short and intracapsular. The latter condition seems to be the rule in the ciliary ganglion of man.

It appears, then, that the autonomic cranial ganglia of the sheep contain cells which are allied in their morphological characters with those of the mammalian sympathetic ganglia. Both are characterized by long dendrites which penetrate the cell capsules. In the possession of these extracapsular dendrites, however, the cranial cells of the sheep differ from the elements of

corresponding ganglia in human beings. In man, as Sala ('10) and v. Lenhossék ('10) have shown for the ciliary, and Müller and Dahl ('10) for the remaining autonomic cranial ganglia, as well as the ciliary, the dendrites are contained within the cell capsules. They are often bent and branched, and may run parallel with the surface of the cell-body for considerable distances, but they do not, as in the sheep, break through the capsular wall.

The terminations of preganglionic fibers

In the ciliary, sphenopalatine, otic and submaxillary ganglia of the sheep the preganglionic fibers terminate in end nets of fine, varicose fibrils embracing the cell-bodies of the postganglionic neurones (figs. 5, 6, 7, 8, 9, 10). These end nets lie inside the cell capsules, and are at places in direct contact with the surfaces of the cell-bodies. We have, therefore, in the cranial autonomic ganglia essentially the same conditions in respect to synapses that obtain in the vertebral and prevertebral ganglia of the sympathetic subdivision of the system. Here the presence of subcapsular end nets has been shown by a number of investigators, notably by Huber ('99), who succeeded in differentiating these terminations with methylene blue in all classes of vertebrates from fishes to mammals.

When examined in detail under high powers of the microscope, the pericellular plexuses are seen to arise through the terminal branching of one or more preganglionic fibers. The largest number observed was four in the ciliary ganglion (fig. 5). Such fibers perhaps result from the division of a single preganglionic neurite, or they may be the terminal portions or collaterals of two or more distinct neurites. In all cases the fibers are non-medullated near the end nets, but when the conditions are favorable for following them, they may be traced, in the opposite direction, into bundles of fibers with thin medullary sheaths.

The fibrils of which the pericellular plexuses are composed show many varicosities. These vary in size and are distributed irregularly along the course of the fibrils. Sometimes they are terminal in position, i.e., they occur at the tips of short free-ending

branches, as is well shown in figure 6, *A*. The varicosities and intermediate portions of the fibrils are often in direct contact with the surface of the cell-body, but they are also found in the space between the latter and its surrounding capsule.

The pericellular plexuses are veritable networks. Anastomoses between the fibrils are frequent, and at the nodes varicosities usually occur. This reticulate condition can be accounted for consistently with the outgrowth doctrine of the development of nerve fibers by assuming that the growing preganglionic neurite, on reaching the postganglionic cell-body, divides tree-like into a number of terminal fibers. These embrace the cell-body on all sides, and, meeting with one another here and there, fuse to form the continuous network.

Although all the end nets are of essentially the same character in the autonomic cranial ganglia of the sheep, they vary in complexity. In the same ganglion one may observe such comparatively simple endings as are shown in figures 8 and 10, and such complete and intricate networks as those of figures 6 and 9. In the drawings only the fibrils and varicosities on the upper sides of the cell-bodies have been represented. By focussing through the transparent ganglion cells the continuations of the nets may usually be seen on the surfaces away from the observer.

In my experience with methylene blue used according to the injection method the end nets of the sphenopalatine ganglion have proved the most difficult to stain satisfactorily. Very frequently the varicosities have been colored a deep blue without affecting the delicate fibrils which connect them. The result gives to the cell-body enclosed by the net a peculiar, spotted appearance. Figure 7 represents a pericellular plexus of the sphenopalatine ganglion in which many of the communicating fibrils are invisible, although some of the varicosities on their course have taken the stain.

In the introductory references to the literature dealing with cranial autonomic ganglia mention was made of the pericapsular 'Nervenfasernetze' demonstrated through Bielschowsky staining by Müller and Dahl ('10) in a single preparation of the sphenopalatine ganglion of man. These bundles of intertwining fibers

are concentrically arranged about the capsules of the ganglion cells. From them a few centrally directed offshoots ending in knobs are traceable through the capsular walls to the surfaces of the underlying cell-bodies. That these extracapsular, nest-like structures are not identical with the intracapsular end nets described above is clear from their position, and the inter-relations of their fibers, which do not appear to anastomose. They are doubtless bundles of preganglionic neurites on the way to their subcapsular terminations, the fibers given off to the cell-bodies being the terminal portions of such neurites. The latter, as has been said, end in knob-like swellings which are in contact with the ganglion cells. Such simple end organs may, in the human sphenopalatine ganglion, put the preganglionic neurites in communication with the postganglionic neurones. However, the foregoing results with methylene blue staining in the sheep raise the question if these knobs are not, in reality, the first varicosities of an intracapsular end net, the remainder of which has not been differentiated by the Bielschowsky technique.

SUMMARY

The sphenopalatine, otic and submaxillary ganglia of the sheep contain multipolar cells with long, slender, frequently branched dendrites, which extend for considerable distances beyond the limits of the cell capsules. They resemble in these particulars the ordinary type of mammalian sympathetic cells.

In the ciliary ganglion the only cells in which processes were clearly differentiated by methylene blue possessed each a single, heavy, branched dendrite.

In all the cranial autonomic ganglia (ciliary, sphenopalatine, otic, submaxillary) the preganglionic neurites terminate on the cell-bodies of the postganglionic neurones in subcapsular, pericellular end nets of fine varicose fibrils. These endings are similar to those of preganglionic fibers in the vertebral and prevertebral ganglia of the sympathetic system.

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PLATE 1

EXPLANATION OF FIGURES

The drawings are camera lucida tracings of ganglion cells stained with methylene blue, and seen under a 2 mm. oil immersion objective. The dotted lines indicate the positions of the cell capsules.

1 Cell from the submaxillary ganglion. *A*, neurite (?).
2 Cell from the otic ganglion.
3 Cell from the sphenopalatine ganglion.
4 Cell from the ciliary ganglion.


1
2
A
3
4

PLATE 2

EXPLANATION OF FIGURES

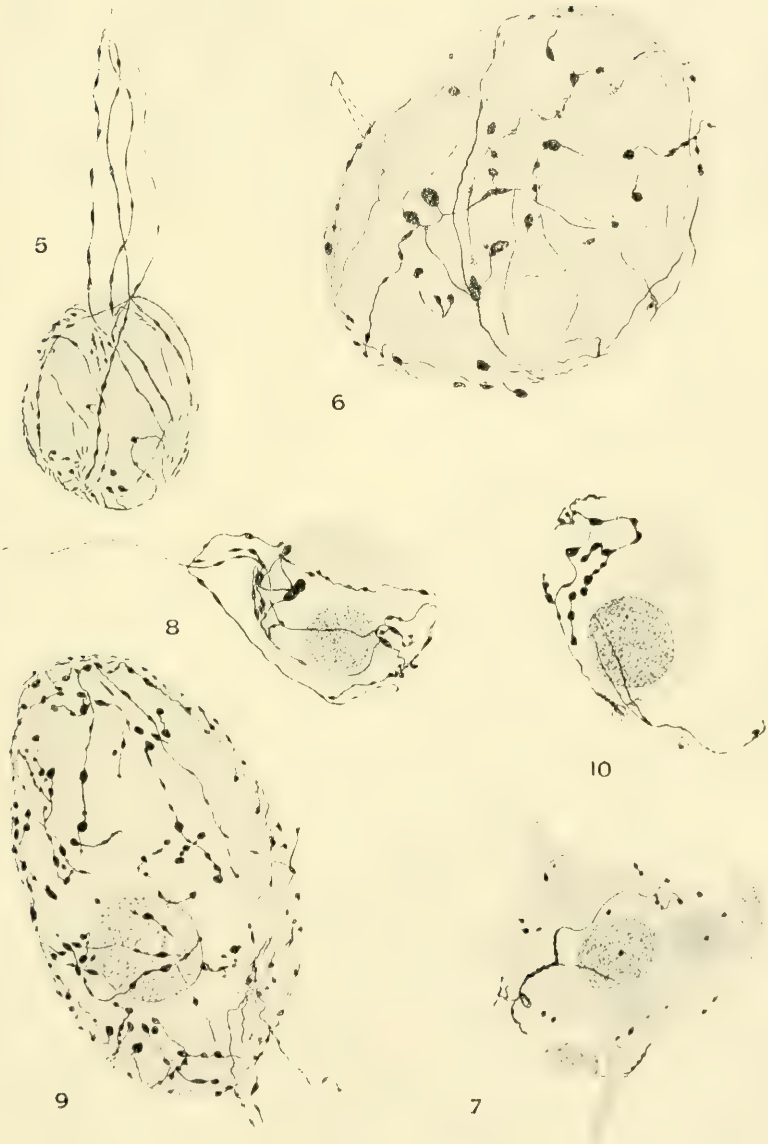
The drawings show the terminations of preganglionic fibers on the cell-bodies of postganglionic neurones. All are from methylene blue preparations seen under a 2 mm. oil immersion objective. The figures have been drawn to the same scale with the aid of the camera lucida, which has been used as far as practicable in reproducing the details of the end nets. Only the fibrils covering the upper surfaces of the cell-bodies have been represented.

5 and 6 End nets from the ciliary ganglion. *A*, terminal varicosities.

7 End net from the sphenopalatine ganglion, incompletely stained. The out line of the cell capsule is shown with two nuclei.

8 and 9 End nets from the otic ganglion.

10 End net from the submaxillary ganglion.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THE CEREBRAL GANGLIA OF THE EMBRYO OF RANA PIPIENS

F. L. LANDACRE AND MARIE F. McLELLAN

From the Zoological Laboratory of the Ohio State University

ELEVEN FIGURES

CONTENTS

INTRODUCTION

A description of the cerebral ganglia of the frog embryo is undertaken as a preliminary to the study of the mode of origin of these ganglia.

This might seem superfluous in view of Strong's ('95) excellent work on the same subject but Strong's work was pioneer in nature and since it was published there have appeared a number of excellent papers on the cerebral nerves and ganglia in Amphibia, particularly those of Coghill ('02) and Norris ('08) in addition to numerous papers on other Ichthyopsida. While most of these papers confirm in general the analysis established by Strong, particularly for the composition of the trigemino-facial complex, there are certain features in the composition of the glossopharyngo-

vagal complex of ganglia that are not as clear as could be desired in Strong's work and do not show the resemblance to the type of composition of ganglia usually found in the Ichthyopsida.

Since the V and VII complex conforms so closely to the type for Ichthyopsida, one would naturally expect the IX and X complex to be equally true to type, because the V and VII is usually the more highly developed and almost invariably has its ganglionic components more closely fused than IX + X in any given stage. An examination of embryos younger than that plotted by Strong shows the IX + X ganglionic complex to be really much more typical and not so highly fused as shown on Strong's plot, thus enabling one to make a much more detailed analysis.

MATERIAL

The series of embryos from which two were selected for plotting consists of eighty-six stages taken from one lot of eggs of Rana pipiens. The first fifty-three stages were taken at intervals of two hours beginning six hours after laying. From the fifty-third stage up to the eighty-fifth the intervals were less regular, ranging from one and three-quarter hours to nine hours, the average being less than five hours.

Out of this series number seventy-two, 8 mm. in length ($171\frac{1}{2}$ hours old) and number eighty-six were chosen for plotting. Number eighty-six is 135 hours older than number seventy-two, being $306\frac{1}{2}$ hours old and 10 mm. in length. In addition to this series, a 25 mm. tadpole of Rana pipiens was studied and also a 35 mm. series kindly loaned by Professor Herrick. Neither of these last two were plotted, but they were carefully compared with the 8 mm. and 10 mm. embryos plotted. Stage 35 mm. seems to be similar in all essential respects to Strong's plot.

THE TRIGEMINO-FACIAL COMPLEX

In the trigemino-facial complex (fig. 1) one point only needs to be emphasized, namely, the distinctness of the profundus ganglion. This ganglion, which in earlier stages is much more isolated than in the 8 mm. embryo, though not entirely distinct, occupies

the dorsal and mesial portion of the ganglion. It extends ventrally as a distinct ganglionic mass about two-thirds of the total dorso-ventral diameter of the combined Gasserian and profundus. The ramus ophthalmicus (*O. V.*, fig. 1) seems at this time to come entirely from the profundus portion, though doubtless there are fibers in this ramus derived from the ventral portion or Gasserian ganglion also. This nerve evidently contains later, if not at this time, the representatives of both the ophthalmic V and of the opththalmicus profundus of the ganoids. The distinctness of this ganglion confirms the position taken by Wilder (92, p. 172) and confirmed by Strong ('95, p. 173), that the ophthalmicus profundus nerve has fused with the ramus ophthalmicus V and that probably in higher types the profundus ganglion is fused with the Gasserian.

The infra-orbital trunk arises from the ventral border of the Gasserian ganglion and immediately splits into the r. maxillaris and the r. mandibularis. Smaller twigs described by Strong could not be positively identified. The apparent difference between the point of origin of nerves in this complex in the 8 mm. tadpole as compared with Strong's description, is due to the fact that in the older tadpole the head becomes flat, thus altering the position of the origin of nerves.

Aside from the difference noted below, the ganglia and nerves of the 8 mm. tadpole conform closely to the description of Strong, whose findings we confirm in every detail except that some of the more minute branches could not be located in our material. It should be noted here that Strong's nomenclature is followed throughout in this paper.

The most striking feature in the arrangement of the V + VII complex is the dorso-ventral elongation as compared with similar stages in Ameiurus (Landacre '10) and Lepidosteus (Landacre '12). This is probably due to the fact that it is crowded between the eye and the ear so that the long axis of this ganglion as well as of IX + X lies at right angles to the long axis of the body. It will be noticed in figure 1 that the special visceral or gustatory portion of the geniculate ganglion could not be identified. This is in marked contrast to Lepidosteus at a similar stage of growth.

THE GLOSSOPHARYNGEAL GANGLION

The visceral portion of the IX ganglion is quite distinct from the X (figs. 1, 2, 3 and 7). It lies ventral to the ear capsule, its anterior end reaching almost to the anterior border of the ear capsule. The ganglionic cells disappear posteriorly at the level of the middle of the capsule and the ganglion here dwindles into a fibrous root which arches around under and behind the capsule and at the level of its posterior end ascends to enter the medulla at the same level as that at which the roots of the X enter it. It was not possible in either the 8 mm. or the 10 mm. stages to identify a special visceral, or gustatory portion, in the visceral IX, although a more careful study of earlier stages leading up to these might enable one to identify it.

The anterior end of the ganglion at this stage abuts against the posterior end of the thymus gland which consists of a dense mass of lymphocytes, oval in shape and lying at the same level dorso-ventrally as the glossopharyngeal ganglion. In stages earlier than that figured (figs. 3, 7, *T.G.*) the ganglion overlaps the gland to a greater extent, usually lying on the dorsal surface of its posterior end. These relations are important because of the fact that in early stages it is difficult to distinguish between the gland and the ganglion histologically, and further, on account of the fact that the ramus lingualis of the IX lies on the outer (lateral) surface and the pharyngeal ramus lies on the inner (mesial) side of the gland.

At the level of the middle of the ear capsule and at the posterior border, situated sometimes on the dorsal portion of the root of the IX sometimes surrounding the root, is a small lateralis ganglion which we have designated the lateralis IX ganglion. This ganglion, so far as we know, has not hitherto been identified as a distinct lateralis ganglion in the frog. Strong, however ('95, p. 144), correctly homologizes the nerve arising from this ganglion (the supra-temporalis) with a lateral line nerve of the IX in fishes. This is undoubtedly correct, since the ganglion has a separate existence from early stages and is only accidentally and quite variously related to the lateral line ganglia of the X.

The exact relation of the various ganglia in the IX and X as figured in plots 1 and 2 to the ganglia shown in Strong's plot is not always easy to determine.

There can be little doubt, however, that the glossopharyngeal ganglion is identical with ganglion *C* of Strong's plot which occupies the extreme anterior end of the IX and X complex. It is hard to conceive of the IX ganglion of our plot being combined with X so as not to bring the IX into the position occupied by ganglion *C*.

The appearance of the IX ganglion in plots 1 and 2 is strikingly similar to that of Menidia (Herrick '99), and of Ameiurus (Landacre '10) and Lepidosteus (Landacre '12) of approximately the same stage.

In the 8 mm. stage there is only one nerve arising from the visceralis IX. This is the ramus lingualis of Strong and runs forward on the outer surface of the thymus gland. In the 10 mm. stage (fig. 2) there are two rami arising from the anterior end of the ganglion, the larger one running down and forward on the outer surface of the thymus gland, the ramus lingualis, and a smaller, the ramus pharyngeus, which runs down and forward on the inner surface of the thymus gland. We have been able to detect no trace of the ramus communicans IX ad VII at this stage in either the 8 mm. or 10 mm. embryo.

In the 35 mm. larva there are three nerves arising from the anterior end of the IX ganglion. Two of them, corresponding to the ramus pharyngeus and the r. lingualis of Strong's plot, undoubtedly arise from the cells of the ganglion, since the fibrous bundles disappear in the ganglion. The third nerve corresponding to the communicating nerve from IX to VII apparently does not arise from the ganglion but runs back on the dorsal surface of the ganglion without diminishing in size and passes into the X ganglionic complex. Its behavior in passing the visceral IX ganglion furnishes strong evidence that it is probably not visceral but purely cutaneous as Strong describes it.

Of the two visceral nerves, the ramus lingualis is much the larger. It arises from the anterior end of the ganglion and pursues a course downward and forward on the outer surface of the

thymus gland. The ramus pharyngeus pursues a similar course downward and forward on the mesial surface of the thymus gland and is distributed to the roof of the pharynx. The ramus communicans IX ad VII leaves the dorsal surface of the IX ganglion at its anterior end and pursues a course forward and downward on the mesial surface of the thymus gland which it leaves finally and, swinging towards the middle line of the body, joins the hyomandibular VII at the posterior border of the eye capsule.

The root of visceral IX can be followed with ease on both the 8 mm. and 10 mm. larvae back to the point where it becomes fused with that of visceral X (figs. 1 and 2, *R.IX*). At this point there is not sufficient difference between the fibers of various roots to enable us to follow them through the ganglion with absolute certainty. The apparent course is indicated in figures 1 and 2 and at the point of emergence from the X ganglion to enter the brain the arrangement with one exception agrees identically with the description of Strong. Strong describes five roots entering the brain from the X, but up to the 10 mm. stage only four can be found. The fifth of Strong's nomenclature is a pure motor root and is probably in our plot combined with the fourth.

THE LATERALIS IX GANGLION

This is a small ganglion situated on the root of the IX nerve just posterior to and at the level of the middle of the ear capsule (figs. 1, 2, 5 and 9, *L.IX*). The position is somewhat variable. In its early stages it has no connection with the lateralis X ganglion. It does, however, in the later stages become fused more or less with either the dorsal lateralis or the ventral lateralis. Its relation with the root of IX is constant, always being in contact with it. It may lie dorsal, ventral, or posterior to this root, but its one constant relation is with the IX. The fact that in its earlier stages it is completely detached from X leaves little doubt that, while it later becomes fused with X, it is really a lateralis IX ganglion and does not belong morphologically to X. The enormous size of the auditory capsule, resulting in crowding the root of the IX back till it joins the X, and the large size of the lateralis X ganglion account for its later fusion with these ganglia. This

ganglion is heavily pigmented like the lateralis ganglion on VII and X. As to its relation to the lateralis ganglion of Strong's plot, we are uncertain. As we shall show later, Strong has apparently overlooked one of the lateralis ganglia on X owing probably to the extent of the fusion of the IX + X complex. Since the lateralis IX may sometimes combine with the dorsal lateralis X, which is probably ganglion *A* of Strong's plot, and sometimes with the dorsal border of ventral lateralis X, which seems to be absent as a distinct ganglion in Strong's plot, it probably corresponds to the ventral border of Strong's ganglion *A*. This ganglion is composed, however, as Strong states, of two distinct ganglionic masses, a general cutaneous, the 'jugular,' and a lateralis X. If it unites permanently with ganglion *A*, it is with the dorsal lateralis X.

There is one nerve arising from this ganglion. It is quite small and pursues in a general way the same course as the auricularis X, being quite separate from it, however, throughout its whole course. It arises from the ventral and anterior end of the ganglion and runs forward around the border of the posterior end of the auditory capsule to reach the epidermis. It is applied very closely to the capsule and is, of course, displaced backwards by the later growth of the auditory capsule. This nerve corresponds to the ramus supratemporalis of Strong's plot. The root of this nerve is difficult to follow but seems to enter the brain along with the lateralis roots of X. From the ganglion it follows a course dorsally and posteriorly along the dorsal surface of dorsal lateralis X, where it seems to join the fibers of this ganglion (figs. 1 and 2).

THE VAGUS GANGLIA

The vagal ganglionic complex consists of five more or less distinct ganglionic masses (figs. 1 and 2). With these is associated in position, as indicated in the preceding section, the lateralis IX ganglion. These five ganglia fall into three well defined groups. First, the most dorsal and proximal portion consists of two ganglia; the dorsal lateralis (*D.L.X.*) and the jugular or general somatic (*Cu.X.*). Of these two, the dorsal lateralis is lateral in position and the jugular is mesial in position. They are of nearly equal

length dorso-ventrally, but the jugular extends farthest forward and the dorsal lateralis farthest posteriorly.

The second group (figs. 1 and 2) consists of two ganglia, the ventral lateralis (*V.L.X.*) and the second division of visceral X (*G.V.X*[2]). This second group lies directly ventral to the first group. In this second group the ventral lateralis is lateral and the visceral ganglion is mesial. These two ganglia are approximately equal in their dorso-ventral length but antero-posteriorly the visceral is broader.

The third group (figs. 1 and 2, *G.V.X*[1]) consists apparently of two branchial ganglia fused, since two branchial nerves arise from it, but is treated here as one ganglion. It occupies the same level dorso-ventrally as the second group, but in general shape is much like the IX ganglion. Its long axis, unlike the two preceding groups, is in the anterior posterior plane and at its posterior end it is fused with the second division of visceral X.

THE DORSAL LATERALIS X

This ganglion (figs. 1, 2, 6, 10 and 11), as mentioned above, occupies the lateral portion of the proximal division of X. It is also the most dorsal portion of the ganglionic complex. It is somewhat elongated dorso-ventrally, not reaching so far forward as the jugular, but extending considerably posterior to any other portion of the X. It begins abruptly in a rounded anterior end and diminishes in size as one reads posteriorly, where the most dorsal of the rami laterales take their origin at the extreme posterior end of the ganglion. The ventral border of this ganglion is in contact in the later stages, but not in the earlier stages, with the dorsal portion of the ventral lateralis X. The resemblance of this ganglion to the lateralis X ganglion in Ameiurus and Lepidosteus (Landacre '10 and '12) at similar stages of growth is striking. The large size of its cells, its position, its clean-cut boundaries and the extension of its posterior border beyond the posterior limits of the remaining ganglia mark it at once as the same ganglion. Its position with reference to the jugular and visceral X is the same in all three embryos.

The relation of this ganglion to the lateralis ganglion of Strong's plot is doubtful. Strong figures and describes apparently only one lateralis ganglion in the X and locates it in ganglion *A*. If the dorsal lateralis of our plot corresponds to Strong's ganglion *A*, there seems to be no homologue of our ventral lateralis in Strong's plot. Our ventral lateralis ganglion is quite distinct in both 8 mm. and 10 mm. embryos but in later stages seems to become more closely fused with the dorsal lateralis X. It is very difficult to determine the limits between the two ganglia in a 35 mm. tadpole. In the 35 mm. tadpole, where the general arrangement of the ganglia and nerves is quite similar to their arrangement in Strong's plot, the dorsal lateralis X is still the most dorsal and posterior portion of the complex.

Dorsal lateralis X in both 8 mm. and 10 mm. embryos gives rise to one nerve. This nerve arises from the extreme posterior end of the ganglion and pursues a course directly backwards as in Lepidosteus, Menidia and Ameiurus. This nerve corresponds to the most posterior R. lateralis, (*1*) of Strong's plot, which curves round behind the auditory capsule before leaving the ganglion. It splits into two divisions as Strong indicates. Its mode of origin and the general position of the ganglion leave no doubt that it corresponds to the ramus lateralis of Ameiurus and Lepidosteus. It also corresponds to the ramus lateralis superior and its dorsal branch in Amblystoma (Coghill '02), and to the lateralis medialis et dorsalis of Amphiuma (Norris '08).

The root of this ganglion (figs. 1 and 2), which enters along with the root of the ventral lateralis ganglion and of lateralis IX, is the most anterior of the roots of X as figured in Strong's plot.

The sympathetic ganglia are discussed here briefly on account of the proximity of the ganglion sympatheticus cervicale of Strong's plot (Strong '95, plate 12, *gang. sym.*) to the proximal part of the X complex. According to Gaupp ('96), there is no sympathetic ganglion in the adult frog occupying a position so close to the X as indicated in Strong's plot.

The adult frog has a sympathetic ganglion on the second spinal nerve, the sensory part of the first spinal nerve of the 10 mm. embryo being absent in the adult. From this first sympathetic

ganglion a sympathetic cord extends forward and enters the X complex, from which a second sympathetic nerve runs forward to unite with the trigemino-facial complex. The connection between the glossopharyngo-vagal ganglion, the so-called jugular of the adult, and the trigemino-facial is intracranial. In the 10 mm. embryo there are sympathetic ganglia on both the first and second spinal nerves in the usual positions. The sympathetic ganglion on the first spinal nerve is quite small, as is also the sensory ganglion of this nerve. The sympathetic ganglion on the second spinal nerve is, on the contrary, quite large. This, as stated above, is the first sympathetic of the adult. A careful examination of our whole series of embryos reveals no sympathetic ganglion in the region of the X. In fact, up to our latest stage and even in a 35 mm. embryo it is not possible to follow the sympathetic nerve from the second sympathetic into the X as a continuous cord and we have been unable to follow with certainty the intracranial connection between X + IX and V + VII ganglia.

These facts indicate that the sympathetic ganglion and cords are in a very immature condition in a 10 mm. embryo and even in a 35 mm. embryo are difficult to follow in detail.

In a 10 mm. embryo the first spinal ganglion is situated 22 sections posterior to the posterior end of lateralis X and the second spinal ganglion is 43 sections posterior to this point, so that the first sympathetic ganglion of a 10 mm. embryo which becomes the first ganglion sympatheticus cervicale is back of the posterior end of lateralis X, a distance equal to the total anterior posterior length of IX + X. The change from this condition to that figured by Strong, if he has correctly located this ganglion, can only be accounted for by the shifting backwards of the IX + X complex by the enlargement of the auditory vesicle.

THE VENTRAL LATERALIS X

This ganglion (figs. 1, 2, 6, 10, *V.L.X.*) occupies, as indicated above, the lateral position in the most distal and ventral division of the second group of ganglia. It is elongated dorso-ventrally; at its dorsal border it is in contact, particularly in later stages, from 10 mm. on, with the ventral end of dorsal lateralis X and

sometimes with lateralis IX. The ventral border of the ganglion diminishes in size and finally gives rise to a lateral line nerve which passes out with a visceral nerve of the X.

The mesial border of the ganglion is more or less closely fused with the lateral surface of visceral X but can always be distinguished from it by the larger size of its cells and by the fact that the cells are always heavily pigmented. The position of this ganglion in Strong's plot is difficult to determine. Our series is not complete from 10 mm. to the 35 mm. embryo. The ganglion seems to shift its position proximally and join the dorsal lateralis ganglion, although not completely, since there are lateralis cells distributed along the dorsal surface of the visceral ganglion in the 35 mm. stage. It must be identified for the present, provisionally, in part, with the ganglion *A* of Strong and in part with the general position of his ganglion B^1 and B^2, to which he does not attribute lateralis cells. The ganglion is not distinct in either Amblystoma or Amphiuma, although Norris ('08) shows in Amphiuma a rather sharp division between the portion of the ganglion from which his ramus medialis et dorsalis and that from which his ramus lateralis ventralis arises. The former probably corresponds to the dorsal lateralis ganglion of our plot and the latter to the ventral lateralis. One nerve arises from the ganglion. It springs from the extreme ventral end of the ganglion and corresponds to the ramus lateralis (5) of Strong's plot. It also corresponds to the ramus inferior (*Li.*) of Amblystoma (Coghill '02) and to the ramus lateralis ventralis (*Lat. V.*) of Amphiuma (Norris '08).

In the embryo frog, as well as in Amblystoma and Amphiuma, this nerve passes out of the ganglion in conjunction with visceral fibers. This is not so evident in Strong's plot, although his Ramus visceralis does come out of the ganglionic complex at the same point but the two trunks are separated up to the ganglionic mass. The conditions in our embryos are quite similar to the plots of Coghill and Norris, where the lateralis and the visceralis trunks pursue a similar course for some distance; at least one of the visceral rami is closely associated with the lateralis ventralis. The root of the ventral lateralis X passes dorsally to enter the brain with the roots of the dorsal lateralis X (figs. 1 and 2).

THE GENERAL SOMATIC X (JUGULAR)

The somatic X ganglion (figs. 1, 2, 6, 10, *Cu.X.*) occupies the mesial position in the proximal mass, or first division of the X ganglion. It is in the 8 mm. and 10 mm. embryos entirely outside the cavity in which the medulla lies. As the head becomes broader and the whole X complex assumes a position more horizontal, general somatic X comes to lie ventral to the dorsal lateralis X and also shifts its position somewhat more proximal so that in a 35 mm. embryo its anterior end is intracranial, the remainder of it lying in the jugular foramen. This shifting of position gives the ganglion the general position occupied by the jugular ganglion in some of the fishes, i.e., intracranial. It extends further forwards than the dorsal lateralis X, but does not reach farther than the middle of that ganglion posteriorly. Its ventral border is usually in contact with visceral X, while its lateral, and later its dorsal border, are in contact with the mesial surface of dorsal lateralis X. The cells of this ganglion are smaller than those of either lateralis X but not so closely packed as those of visceral X. The ganglion is quite large, much larger than the jugular at similar stages in Ameiurus or Lepidosteus.

There is little difficulty in locating this ganglion in Strong's plot. It represents a portion of his ganglion *A*. It is quite distinct in all stages of the embryo up to and including the 10 mm. stage. The most conspicuous nerve arising from this ganglion is the ramus auricularis, (2) of Strong's plot. He figures it as a pure general cutaneous nerve. It seems to be such in the embryo, but arises from the X ganglion in both Amblystoma and Amphiuma as a mixed nerve containing both general cutaneous and lateralis fibers. Its point of origin from the jugular ganglion is so close to the dorsal lateralis that there may possibly be lateralis fibers in it. Strong's interpretation of it as a pure general somatic nerve seems to hold however for the embryos we have studied.

The ramus auricularis arises from the anterior border of the ganglion at about the middle of its dorso-ventral extent and arches forward and outward to curve around the posterior border of the auditory capsule. A comparison of figures 1 and 2 will show

that the ramus auricularis X takes its origin from the jugular ganglion farther posterior in a 10 mm. embryo than in the 8 mm. stage. A careful examination of twenty series lying between the 8 mm. and the 10 mm. stages shows that in all but one of them the ramus auricularis arises as in the 8 mm. stage In the 10 mm. stage this nerve arises posterior to the fibrous bundle connecting the two ventral ganglia of X to the two dorsal ganglia. This fibrous bundle passes lateral to the jugular ganglion (figs. 6 and 10) and it is probable that it is the active factor in determining these relations, i.e., it may form anterior or posterior to the point of exit of the ramus auricularis from the jugular ganglion.

The differences between the fibers of different components is not sufficiently great to enable one, at this stage, to follow the general cutaneous fibers into all the nerve trunks figured by Strong. The size of the ganglion is entirely sufficient to furnish, in addition to the ramus auricularis, the rather large bundles running out in two of the branchial rami and the ramus communicans IX ad VII. In describing this last ramus attention was called to the fact that, while it enters the anterior end of the glosso-pharyngeal ganglion, it runs past that ganglion and follows the root of the IX to enter apparently the jugular. Questions concerning the composition of mixed nerves are not easily settled on the material of the age used in this paper. Fortunately most nerves, even when mixed in the adult and in later embryonic stages, are likely to be pure and arise separately from a distinct ganglionic mass if the proper stage is studied. This is true of the ramus auricularis, but is not true of the other rami arising from the jugular ganglion, so that our findings as far as they go confirm those of Strong with respect to the rami arising from this ganglion.

THE SECOND VISCERALIS X

This ganglion (figs. 1, 2, 6, 10, $G.V.X^2$) occupies the mesial portion of the distal division of the vagus complex. On its dorsal and proximal border it is in contact with the jugular and on its lateral, and later on its dorsal surface, it is in contact with the ventral lateralis. On its anterior border it is in contact with the

branchial X (figs. 1 and 2, $G.V.X^1$). Its longest diameter, like all other members of the vagus complex except the jugular and branchial, is the dorso-ventral. It projects somewhat further caudad than the ventral lateralis but not so far as the dorsal lateralis.

The position of this ganglion in Strong's plot cannot be determined with certainty. The portion of the X complex from which the visceral rami arise is represented as a mesial projection attached to the main ganglionic mass at the level of the ganglion B^2. It is labelled 'ramus visceralis 3' but is described by Strong as containing ganglion cells. There seems no doubt that the ramus visceralis (3) of Strong's plot represents the apex of our visceral ganglion, but whether the proximal part of our visceral ganglion is represented by B^1 or B^2 of Strong's plot is uncertain. It is probably represented by B^1, since the rami branchiales seem, on his plot, to come from B^2. The comparison on this basis harmonizes the two plots, since in the earlier stages represented in our plot the branchial nerves come from the branchial ganglion. The relations in the 10 mm. embryo plot are quite clear and barring the reduction in the number of branchial nerves are quite typical.

The visceral nerves arising from this ganglion emerge from its ventral apex. There are two chief divisions at this stage. The more ventral arises in conjunction with the ramus lateralis ventralis (*L.X.V.*, figs. 1 and 2) and the other arises somewhat more proximally and pursues a course directly posterior. This last branch is undoubtedly the ramus intestinalis (figs. 1 and 2, *V.X.*). As to the composition of the first, the branch arising with the lateral line ramus, there is less certainty. It probably is not purely sensory, but contains a large motor ramus, (4) of Strong's plot. But there are certainly visceral fibers in it also, probably supplying the fourth gill.

BRANCHIALIS X, OR FIRST VISCERALIS X

This ganglion (figs. 1, 2, 4, 8, $G.V.X^1$) stands in sharp contrast to the remaining portion of X both in its shape and in its position in the body. It resembles both in shape and position the glossopharyngeal ganglion. Its long axis is parallel to the long axis of the body. It lies under the auditory capsule like the IX and not behind it like the remainder of the X. Its general shape is that of an elongated S-shaped column of cells, free at its anterior border but attached at its posterior border to visceralis X^2. This attachment occurs at the middle or upper third of the anterior surface of the visceralis X. The anterior end of this ganglion lies under the posterior end of the auditory vesicle. From its anterior end, where a branchial nerve arises, it gradually increases in size until it fuses at its posterior end with the visceral X^2.

The position of this ganglion in Strong's plot is represented apparently by ganglion B^2. It is from this ganglion that the branchial nerves emerge in his plot and Strong's ganglion C is, undoubtedly the glossopharyngeal. The only question in doubt is whether branchial X also corresponds to a part of B^1. This seems improbable since B^1 is the only ganglion left on Strong's plot that could represent the visceral X^2. The extent to which the IX + X complex is elongated by the posterior extension of the auditory capsule makes it difficult to follow the shifting of the ganglia, since there is a stage between the time when the ganglia are distinct, as in our plots, and the time when fibrillated paths can be followed, in which it is very difficult to determine ganglionic boundaries and still more difficult to separate components among fibers.

An interesting question arises here as to the homology of the branchial ganglia in the frog with the branchial ganglia of Menidia, Lepidosteus and Ameiurus. In these types there are four more or less distinct branchial ganglia in the vagal complex. In Lepidosteus, which at a similar stage of development most closely resembles the 8 mm. stage of the frog, only one branchial nerve, that for the second true gill, arises from the first branchial X ganglion. The remaining three arise from the ventral border

of the general visceral ganglion, each branchial nerve arising from a ventral prolongation extending downwards towards the appropriate gill. The condition shown by Herrick ('99, text-fig. 5) gives the exact relation of these branchial ganglia in Lepidosteus and Ameiurus, with the exception that the branchial ganglia are a little larger and somewhat more detached than in Ameiurus and Lepidosteus and the general visceral portion seems to be smaller. In the frog there are only two large branchial nerves and they both arise from the same ganglion. This ganglion has the same appearance and morphological relations as Branchial X^1 of Lepidosteus (Landacre '12). The question arises whether the branchial ganglion of the frog represents the branchial X^1 of Lepidosteus, Ameiurus and Menidia or whether it represents two or more branchial ganglia of these types fused. The question can be answered definitely only by careful study of the embryological development of the branchial ganglia and nerves. The condition in the ganoid (Landacre '12) indicates that branchial ganglia other than X^1 are incorporated with the general visceral X, since these ganglia are much smaller than in Menidia and much less distinct. The behavior of the second branchial nerve arising from this ganglion, as will be shown later, indicates that the ganglion that we have called visceralis X^2 really represents the general visceral X of Lepidosteus plus one or more branchial ganglia and that the branchial ganglia of X (figs. 1 and 2, $G.V.X^1$) represents principally branchialis X^1 of Lepidosteus.

As mentioned above, there are two branchial nerves (figs. 1 and 2, $Br.X^1$ and $Br.X^2$) arising from branchialis X. These correspond to the rami branchialis (6) and (7) of Strong's plot. The anterior (6 of Strong's plot) arises from the extreme anterior end of the ganglion and pursues a course downward and forward to the gill. The second (7 of Strong's plot) arises toward the posterior end of the ganglion and runs downward and forward to the gill. This nerve on entering the ganglion, however, is not lost entirely among the ganglion cells, as is the first nerve, but pursues a course backward on the under surface of branchial X until it reaches the anterior border of visceral X. It is, however, in its course diminished in size and it may be possible that some of

its cells of origin lie in the posterior portion of branchial X. It is hardly probable that all of them lie in branchial X, so that until the embryological origin is worked out the problem of the exact morphology of branchial X^1 will have to be left as indicated above, i.e., that a part of the cells from which the second branchial nerve (6 of Strong's plot) originates lie in the visceral ganglion and that the visceral ganglion represents the general visceral ganglion of such types as Menidia, Lepidosteus and Ameiurus, plus the representative, one or more, of branchial ganglia of these types.

SUMMARY AND DISCUSSION

1. The trigemino-facial complex of the frog in stages earlier than those studied by Strong corresponds in all essential details with his analysis except in the greater isolation of the profundus. This ganglion in earlier stages is much more isolated than in the earliest plot given in this paper, but even in that it stands out rather distinctly, indicating its definite character which is lost by incorporation with the Gasserian. In other respects we confirm Strong's account of the V + VII ganglia in the frog.

2. The glossopharyngo-vagal complex of ganglia, on the contrary, if taken in the 8 mm. and 10 mm. stages, shows a much greater degree of simplicity and isolation of its various components than indicated by Strong and furthermore it is much more typical as compared with such types as Menidia, Ameiurus and Lepidosteus.

3. The lateralis components in the glossopharyngo-vagal complex are represented by three more or less distinct ganglia. These are, (a) a lateralis IX situated on the root of the IX behind and at the level of the middle of the auditory vesicle dorso-ventrally; (b) two lateralis X ganglia situated on the lateral surface of the cutaneous and general visceral ganglia respectively. Of these two ganglia, the dorsal lateralis is proximal and the ventral lateralis distal. The dorsal lateralis lies lateral to the jugular, or general cutaneous X, and gives rise to one nerve trunk which immediately after leaving the ganglion splits, giving rise to the most posterior ramus of Strong's plot (dorsalis and medialis of

Coghill and Norris). This ganglion resembles closely in form and position the lateralis X of Ameiurus and Lepidosteus in similar stages of development. It extends considerably posterior to any other ganglion and the nerve arises at the posterior attenuated extremity as in those types.

(c) The ventral lateralis X is the third of these ganglia. It is ventral to dorsal lateralis X in young embryos, but later, owing to the flattening of the head, becomes more lateral in position. It lies lateral to visceralis X^2 in young embryos and later assumes a more dorsal position with reference to visceralis X^2 and also becomes more closely fused with the dorsal lateralis X. It gives rise to one nerve which emerges from the ventral apex of the ganglion (the most anterior lateral line ramus (5) of Strong's plot and the ramus lateralis ventralis of Coghill and Norris).

The presence of two lateral line ganglia on X suggests at once a homology with the condition in VII where there are two lateral line ganglia also. As to the distinctness of this ganglion up to and beyond the 10 mm. stage there can be no doubt. Nor is it doubtful that it is a lateral line ganglion. The size of its cells, their heavy pigmentation and the isolation of the ganglion settles both these doubts. As to the homology with ventro-lateral VII, we are not willing to go farther at present than to gives it a name signifying its composition and position in the X complex. It gives rise to the same component as ventro-lateral VII, it occupies the same relative position, i.e., lateral to a visceral ganglion and in the distal portion of the complex as does ventro-lateral VII. Its nerve also runs out in conjunction with a branchial nerve. If it should prove to have a similar mode of origin to that ganglion there would seem to be no objection to homologizing them. No other fish or amphibian studied so far as we know has a distinct ventral lateralis ganglion. An examination of the reconstructions of Coghill ('02) and of Norris ('08) indicates that probably the same condition will be found in Urodeles, since the lateral line cells extend well down ventrally toward the origin of the ramus lateralis ventralis in both cases.

4. The jugular, or general somatic X, as in other Ichthyopsida above the Cyclostomes, is the only representative of its type in

the IX + X complex. It is situated mesial to the dorso-lateral X and is consequently proximal of the visceral X. It is very large in the tadpole, much larger than in the Lepidosteus and Ameiurus at the same stage of growth and is much farther away from the medulla. In the 35 mm. tadpole, however, it migrates towards the medulla and lies partly, though not wholly, in the jugular foramen.

One nerve arises pure from this ganglion at this stage, the ramus auricularis; others arise also but run out in conjunction with other components and could not be accurately traced. So far as they could be followed their distribution agrees with Strong's description.

5. The visceral ganglia of the glossopharyngo-vagal complex lie in three well defined masses: (a) the glossopharyngeal; (b) the first or most anterior visceral, and (c) the second or most posterior visceral.

(a) The glossopharyngeal ganglion occupies a position ventral to the auditory capsule with its long axis parallel to that of the body. The root is long and curves around behind the auditory capsule to enter the medulla along with those of X. It seems to be a pure visceral ganglion, although a cutaneous ramus, r. communicans IX ad VII, passes out from the anterior end of the ganglion. Its fibers, however, can be traced past the ganglion and apparently enter jugular X, as described by Strong. It was not possible in the stages studied to recognize the special visceral or gustatory portion of this ganglion. Two nerves, the ramus pharyngeus and the ramus laryngeus, arise from the anterior end of this ganglion.

(b) The first visceral or branchial ganglion of X resembles in shape and position the glossopharyngeal. It lies under the posterior end of the auditory capsule with its long axis parallel to that of the body. Its posterior end is not, like that of IX, continued into a fibrous root but joins the cell mass of the second visceral X near its middle region. Gustatory cells could not be recognized in this ganglion in the stages studied. Two large nerves arise from this ganglion, the branchial 6 and 7 of Strong's plot.

The presence of two branchial nerves arising from this ganglion would suggest that it represented two branchial ganglia fused. However, there is some doubt as to whether the fibers of the second branchial nerve arise in this ganglion or partly in this ganglion and partly in the second visceral ganglion.

(c) The second visceral X ganglion, unlike the glossopharyngeal and the first visceral X, lies with its long axis at right angles to that of the body. It gives rise to two chief nerve trunks; one, the ramus intestinalis and a smaller ramus which probably contains branchial fibers. The question as to the exact position of the branchial ganglia in these two visceral ganglia cannot be definitely settled without further examination of earlier stages. A careful study of a close series of embryos will probably show definitely the number and position of the epibranchial placodes and their position in the visceral ganglia, thus determining the number of branchial ganglia in this complex and their location.

6. The failure to distinguish special visceral or gustatory ganglia in Rana in the stages studied is not to be interpreted to mean, of course, that they are absent or cannot be isolated. As mentioned in the introduction, this paper is preliminary to a study of the mode of origin of the cerebral ganglia in the frog. As a matter of fact, epibranchial placodes are present in the stages earlier than 8 mm. and well defined and seem to behave much as they do in Ameiurus and Lepidosteus. It is hardly probable that they will be so distinct or can be followed to so late a stage as in Lepidosteus, since they are not recognizable in the 8 mm. stage. Neither is it likely that the frog will furnish such definite evidence as to the character of placodal ganglia as did Ameiurus, since all nerves arising from the IX seem to contain both general visceral and special visceral fibers, whereas in Ameiurus they seem to contain only special visceral fibers.

7. The results of the present paper emphasize the immense importance of having access to a large number of stages taken at close intervals from the same lot of eggs, if one is to reach safe conclusions in regard to the composition and origin of cerebral ganglia. All ganglia, particularly those derived from the neural crest, in their early stages are more or less ill defined; following

this stage in Lepidosteus, Ameiurus and the frog there is a stage when the ganglia are better defined, have clean cut boundaries and give rise to fibrillated nerves, usually well isolated from each other; following this stage the ganglia fuse together more or less, their nerve trunks combine and they must be isolated largely by the difference in size of their nerve fibers. Evidently the second stage is the one most favorable for determining the number and position of ganglionic components and this stage can be found only when the series is complete and taken at close intervals.

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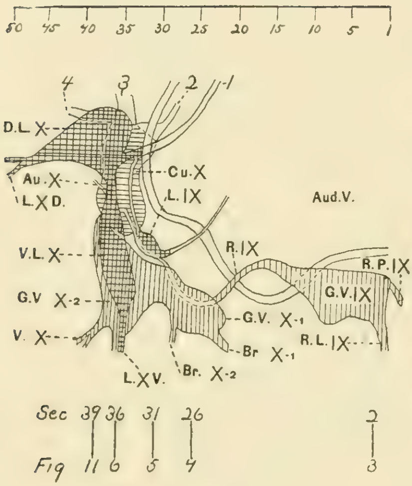
ABBREVIATIONS

Aud. V., Auditory vesicle
Aud., Auditory ganglion
Au. X., Ramus auricularis X.
Br. X[1], First ramus branchialis X.
Br. X[2], Second ramus branchialis X.
Bu. VII., Ramus buccalis VII.
B. V., Blood vessel
Cu. X., Cutaneous or general somatic (jugular) ganglion of X.
D. L. X., Dorsal lateralis ganglion of X.
D. L. VII., Dorso-lateral lateralis ganglion of VII.
Gass., Gasserian or general somatic ganglion of V.
Gen., Geniculate or visceral ganglion of VII.
G. V. IX., Visceral (Glossopharyngeal) ganglion of IX.
G. V. X[1], First division of the visceral ganglia of X; possibly equivalent to two branchial ganglia
G. V. X[2], Second division of the visceral ganglia of X.
Hyo. VII., Hyomandibularis VII.
L. IX., Lateralis ganglion of IX.
L. X. V., Ventral ramus of the lateral line nerve of X = R. lateralis (5) of Strong's plot. Strong '95, plate 12
L. X. D., Dorsal ramus of the lateral line nerve of X = R. lateralis (1) of Strong's plot. Strong '95, plate 12
Man. V., Ramus mandibularis V.
Max. V., Ramus maxillarus V.
Mes., Mesencephalon
No., Notochord
O. V., Ramus ophthalmicus V.
O. S. VII., Ramus ophthalmicus superficialis VII.
Opt., Optic vesicle
Pal. VII., Ramus palantinus VII.
Prof., Profundus ganglion
R. V., Root of V.
R. L. IX., Lateral line root of IX.
R. IX., Root of IX.
R. P. IX., Ramus pharyngeus IX.
R. X. V + C., Visceral + somatic roots of X.
R. L. X., Lateral line root of *X* + IX.
R. X., Root of X.
S. T. X., Ramus supra-temporalis X.
T. G., Thymus gland
V. L. VII., Ventral lateral line ganglion of VII.
V. L. X., Ventral lateral line ganglion of X.
V. X., Ramus visceralis X.

Fig. 1 A flat reconstruction of the V, VII, VIII, IX and X cerebral ganglia in Rana pipiens, 8 mm. in length. The scale at the top of the figures indicates the position and number of sections 10μ in thickness over which the plot extends. General somatic ganglia are shown with horizontal lines, lateral line ganglia with cross hatched lines, and visceral ganglia with vertical lines. Special visceral or gustatory ganglia are not indicated, as their boundaries could not be determined in embryos of this age. $\times$ 100.



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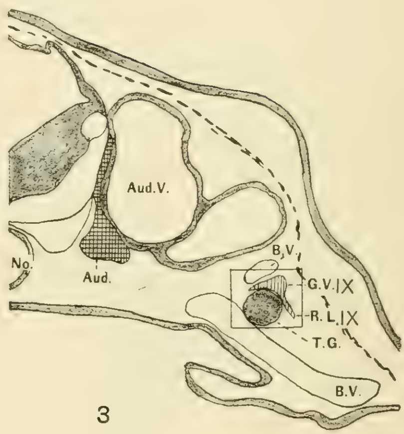

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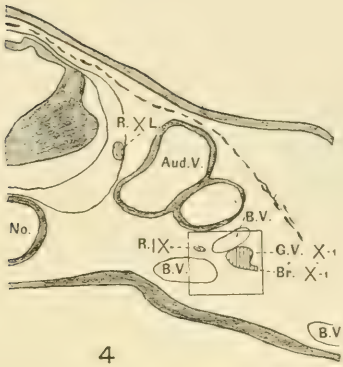
Fig. 2 A flat reconstruction of the IX and X cerebral ganglia of Rana pipiens 10 mm. in length; shading as in figure 1. The scale at the top of the figure indicates number and position of sections 10μ in thickness over which the plot extends. The scale at the bottom of the plate indicates the number of the section and the number of the figure illustrated in succeeding plates. As in figure 1, the special visceral or gustatory ganglia are not indicated. 1, 2, 3, 4 are the first four roots according to Strong's nomenclature (Strong '95, plate 12, fig. (a) and page 135). $\times$ 100.

Fig. 3 A camera outline through the dorsal portion of the right side of the head of Rana pipiens, 10 mm. in length. The section passes through the anterior end of the IX ganglion and the origin of the ramus laryngeus. The position of the section on figure 2 is indicated at the bottom of that figure (Sec. 2). The details of the area blocked out are shown on figure 7. $\times$ 50.



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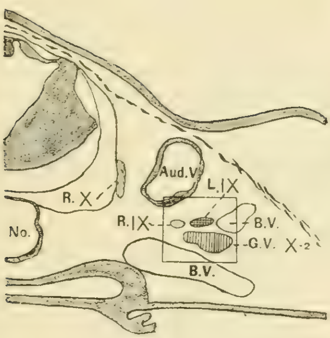

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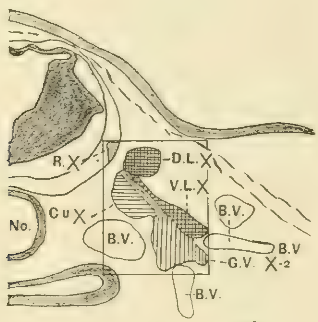

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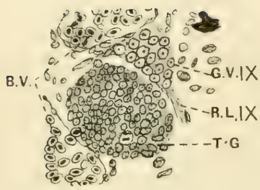
Fig. 4 A camera outline through the dorsal portion of the right side of the head of Rana pipiens, 10 mm. in length. The section passes through the middle of the first portion of the visceral X ganglion ($G.V.X^1$, fig. 2) and the posterior portion of first branchial nerve ($Br. X^1$) of that ganglion. The position of the section is indicated at the bottom of figure 2 (Sec. 26). The details of area blocked out are shown in figure 8. × 50.

Fig. 5 A camera outline of the dorsal portion of the right side of the head of Rana pipiens, 10 mm. in length. The section passes through the lateralis IX (*L.IX.*) and the second visceral ganglion of X ($G. V. X^2$). The position of this section is indicated at bottom of figure 2 (Sec. 31) and the details of the area blocked out are shown in figure 9. × 50.

Fig. 6 A camera outline through the dorsal portion of the right side of the head of Rana pipiens, 10 mm. in length. The section passes through the four posterior masses of the X ganglion. The position of this in figure 2 is indicated at the bottom of that figure (Sec. 36). The details of the area blocked out are given in figure 10. × 50.



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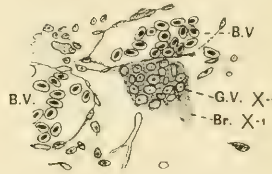

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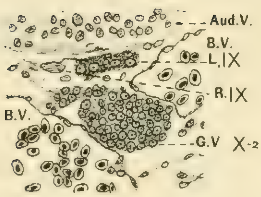

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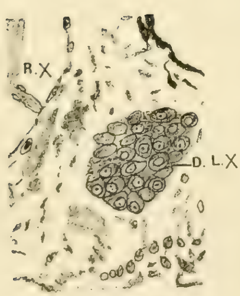

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Fig. 7 Details of area blocked out in figure 3. × 200.

Fig. 8 Details of area blocked out in figure 4. × 200.

Fig. 9 Details of area blocked out in figure 5. × 200.

Fig. 10 Details of area blocked out in figure 6. × 200.

Fig. 11 Details of the lateralis X ganglion. The position of this section on figure 2 is shown at the bottom of that figure (Sec. 39). × 200.

DEGENERATION AND REGENERATION OF NERVE FIBERS

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TWENTY-NINE FIGURES

The series of experiments upon which this paper is based was begun with no idea of taking up the question of the regeneration of nerve fibers, but with the much more restricted purpose of studying the degeneration of the non-medullated fibers of the spinal nerves. After it had been demonstrated that the spinal nerves contain many more non-medullated than medullated fibers (Ranson '11) it was very desirable that the Wallerian experiment should be repeated to determine the direction of degeneration in these fibers. Work had not progressed far, however, before it became evident that observations were being made that had a bearing on the perplexing question of nerve regeneration and made it necessary to enlarge the scope of the investigation while not abandoning its original object.

Since, as has been intimated, we shall be concerned in part with the degenerative and regenerative phenomena in the non-medullated fibers, it will be best to indicate at this point the facts which have been previously ascertained in regard to these fibers.

When ordinary staining methods are used it is possible to see in the spinal nerves only those fibers which possess a myelin sheath, since axons not so covered cannot be differentiated from the connective tissue of the endoneurium. But when

[1] Some of the work, on which this paper is based, was done in the Anatom. biol. Institut, Berlin, the Anatom. Anstalt, Freiburg i. Br. and the Physiol. Institut, Freiburg i. Br. To the directors of these institutes I am indebted for many courtesies.

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these nerves are stained by a modification of Cajal's method, which we have called the pyridine-silver method, very many fine nerve fibers are seen in the endoneurium between the medullated fibers (Ranson '11). These are stained black with reduced silver and are devoid of a myelin sheath, while the medullated axons are light yellow and are surrounded by a colorless sheath of myelin. The non-medullated fibers may run singly but are usually grouped into small bundles. Although no attempt has been made to determine their exact number, it is easy to see that they far outnumber those which are medullated. On tracing these fibers back toward the spinal cord, it is found that, while a certain number can be seen entering the nerve via the gray ramus communicans (Chase, unpublished observations), the vast majority of them enter the nerve by way of the dorsal root. A study of the spinal ganglion (Ranson '12) has shown that these fibers are derived from the axons of the small cells of the ganglion by a *T*- or *Y*-shaped branching. Because of the location of the cells of origin in the spinal ganglion, and because of the characteristic *T*- or *Y*-shaped branching of the axons there need be no hesitancy in regarding them as afferent and not sympathetic in function.

As has been intimated, it was our purpose, when the series of experiments to be reported in this paper was begun, to cut first the peripheral nerve and then, in other experiments, the roots proximal to the spinal ganglion in order to determine the direction of degeneration of these fibers. The division of the sciatic nerve of the dog was undertaken to exclude by the direction of degeneration the possibility that some of the non-medullated fibers might arise from cells located at the periphery (a common location of such cells in invertebrates). In another series of experiments the roots, proximal to the ganglion of the second cervical nerve of the dog, were cut to determine whether any non-medullated fibers arose from cells located in the gray substance of the spinal cord. Since the results of these two series of experiments were complicated by a variety of regenerative changes in the nerve, ganglion and roots, it has seemed best to present the two series of experiments in separate papers.

And the present paper will be concerned only with the results of section of the sciatic nerve.

The majority of those who have worked with the problem of the regeneration of nerves had previously taken a definite position in the contest over the neurone theory and sought in their experiments on nerve regeneration to find evidence which would aid them in maintaining that position. The supporters of the neurone theory believed that the axons were formed embryologically as outgrowths from ganglion cells and were regenerated from the axons of the central stump toward the periphery. On the other hand, the opponents of the neurone theory believed in a multiple origin of the axons from the cells along the course of the embryonic nerves, and described a similar discontinous origin of the new axons in regenerated nerves by a differentiation of the protoplasm formed from the old neurilemma cells. The present writer, having never committed himself in the matter of the neurone theory, was able to take an entirely unprejudiced attitude and was led to his present position as he studied the regenerative phenomena which appeared, unexpectedly and with great clearness, in the preparations of the divided sciatic nerve.

SURVEY OF THE LITERATURE ON THE REGENERATION OF NERVES

Even before the time of Waller the subject had received some attention, but it was he who first recognized the true nature of the degenerative and regenerative processes in nerves. He stated ('52) that that portion of a divided nerve fiber separated from its trophic center underwent complete degeneration and that regeneration took place through an outgrowth of new fibers from the central undegenerated stump. His conclusions were not at once accepted, however, and even to the present day the outgrowth of the new axons from those of the central stump is a matter of debate. It would tax too heavily the reader's patience to review the literature from Waller's time to the present date, but this is made unnecessary by the excellent reviews of the early literature, given in the articles of Howell and Huber ('92) and Stroebe ('95). According to the summary given by Howell

and Huber of the problem as it stood at the time of the writing of their article, three views were then held: (1) According to one view, the axons did not degenerate in the peripheral stump after they had been separated from the central nervous system, so that, after the two ends of a cut nerve had been approximated, it was only necessary for a peripheral axon to fuse with a central axon and for a new myelin sheath to be developed about it. (2) The view most generally current at that time was the view of Waller that the new axons grew out from the central end. (3) But they state that the best of the papers which had appeared in the years immediately preceding their work agreed in stating that the axis cylinder is developed anew in the peripheral end and is connected secondarily to the central end. They add that those who maintained that the new axis cylinders are sprouted from the old ones of the central end did not claim to have seen the process, since the closest study with the staining method then in use showed nothing of the axis cylinder at the point of transition. In the opinion of the present writer, it was just this lack of a satisfactory axon stain and the resulting uncertainty of what was occurring at the transition zone between the ends of a cut nerve that has made it possible for the supporters of the theory of the autogenous regeneration of nerves to maintain their position for so many years.

With the work of Büngner ('91), Howell and Huber ('92), Stroebe ('93) and Huber ('95), the modern work on the regeneration of nerves may be said to have begun. Büngner ('91) saw and clearly described the formation of the nucleated protoplasmic bands which have occupied so prominent a place in the supposed histogenesis of autogenously regenerated nerve fibers. According to him, the nuclei of the neurilemma increase in number and protoplasm accumulates about them. Longitudinal striation appears in the protoplasm of these cells which then become fused into long multinucleated bands. Within these bands the centrally placed, longitudinally striated protoplasm becomes differentiated into a new axon, while in the surrounding protoplasm the myelin sheath is laid down.

Howell and Huber ('92) also saw and described these protoplasmic bands, and, while they were unable to confirm Büngner's observations on the differentiation of axons in them, they regarded them as 'embryonic nerve fibers' capable of receiving and conducting impulses. In case of a failure of the two ends of the nerve to unite, regeneration stopped with the formation of the protoplasmic bands. In case of union of the two stumps, the bands of the peripheral stump fused with those of the central stump; next myelin sheaths were laid down within the bands and new axons grew down into them from the central stump.

Stroebe ('93), using his now well known stain, was able to demonstrate the outgrowth of new axons from the central end, but thought that the myelin sheath was formed as a continuation of the old sheath and accompanied the axon in its forward growth. He was unable to demonstrate any connection between these new fibers and the protoplasmic bands seen by Büngner in the peripheral stump.

Working with Stroebe's method, Huber ('95) was able to secure much clearer pictures of the regenerating nerve fibers than had been seen before, and showed that the axons grew out from those of the central stump and, in some cases at least, entered the substance of the protoplasmic bands of the peripheral stump. He was able to see some which ended abruptly in the protoplasm of the bands, their free ends directed toward the periphery and occasionally presenting bulbous enlargements.

Ziegler ('96) ascribed the origin of the new axons to the neurilemma cells of the central stump and thought that at the time of their first appearance they had no connection with the old axons. Galeotti and Levi ('95), Kennedy ('97), and Wieting ('98) thought that the new axons developed in situ in the peripheral stump. It is clear, therefore, that even before Bethe began his work the opinions of those who had worked at the question from the histological side were about evenly divided. This shows that the histological methods at their disposal were not adequate for the solution of the problem.

Since investigators had failed to show conclusively the nature of the regenerative processes when the two stumps had been

allowed to unite, Bethe ('03) decided to follow the example of Philipeaux and Vulpian ('59) and determine what would occur in a nerve fully cut off from its central connections. He worked with the sciatic nerve of young dogs and to prevent the union of the cut ends he adopted special operative procedures: (1) In some dogs he grasped the sciatic nerve at the great sacrosciatic foramen, tore it out along with the motor roots and spinal ganglia and cut it off in the middle of the thigh. (2) In others he cut the sciatic in the middle of the thigh, left the peripheral stump in place, cut off 3 cm. from the central stump and thrusting it through one muscle, sewed it into another. At that time he considered the second method as reliable as the first. In these ways he believed that he had effectually excluded the possibility of any union between the central and peripheral stump; and in carefully conducted autopsies he was unable to find any trace of such connection in those cases which he considered successful. Also physiological evidence of the absence of connection between the central and peripheral ends was obtained at autopsy in the absence of reflex movements on stimulating the distal stump and the absence of movement in the muscles innervated by the sciatic nerve on stimulation of the central stump.

Under these circumstances, which seemed both anatomically and physiologically to exclude the possibility of any union with the central nervous system, Bethe obtained evidence of regeneration in the peripheral stump. Many medullated fibers were present. Stimulation of the peripheral stump caused contraction in the muscles of the leg but no reflex movements. He also noticed that some time after autogenous regeneration had occurred a slow degeneration set in which caused, after a time, the complete disappearance of all regenerated fibers. This Bethe considers an important point in favor of the autogenous nature of their regeneration, since, he assumes, fibers which have formed central connections do not undergo a second degeneration. In his somewhat limited study of the development of these autogenously regenerated fibers he distinguishes five stages: (1) protoplasmic band formation; (2) differentiation

of these bands into axial strands and granular sheaths 'Achsialstrangfasern'; (3) appearance of fibrils in the axial strands in the neighborhood of the nuclei; (4) fusion of these discontinuously formed fibrils into fibrillar bands; (5) discontinuous formation of a myelin sheath. Bethe maintains that such transformation can occur only in young animals and even then only in a limited number of the fibers. Many fibers in young animals and all in adult animals are incapable of developing beyond the stage of protoplasmic bands without some stimulus from the central stump.

So convincing did these experiments of Bethe appear that they excited the greatest interest and have led to many attempts to secure confirmation of his results. Münzer ('02), Head and Ham ('03) and Mott, Halliburton and Edmunds ('04) presented evidence to show that all new axons in the peripheral stump were outgrowths from the old axons of the central end. But there have appeared two investigations, those of Langley and Anderson ('04) and Lugaro ('05), which not only show that Bethe's conclusions were wrong, but show clearly the fallacies upon which his erroneous conclusions were based. The work of Langley and Anderson ('04) was carried out on kittens and young rabbits. Experiment 5 is typical of their results. The right sciatic nerve was cut high up in the thigh, turned down and sewed into the skin above the knee. After 251 days the sciatic was cut above the first point of section and the femoral nerve was cut near the inguinal ligament. The peripheral sciatic stump, twelve days after the second operation, contained no sound medullated fibers but many degenerated ones. Summing up all their experiments they say:

> We find that all the medullated nerve fibers, which reform in the peripheral end of a nerve, degenerate when the nerves which run to the tissues near the cut end are cut near the spinal cord; in other words, in our experiments all medullated fibers in the peripheral ends of the cut nerves were fibers which had become connected with the central nervous system. If then, autogenetic regeneration of fibers had occurred, every one of them had become connected with the central end of some nerve fiber. On the autogenetic theory this seems to us in the highest degree unlikely.

It is important not to overlook the possibility of fibers growing into the distal stump from other nerves in the neighborhood.

Against the physiological tests of the absence of connection between the two ends of a divided nerve, failure to get reflex movement on stimulation of the peripheral stump or movement in the muscles of the leg on stimulation of the central stump, tests upon which Bethe has always laid the greatest stress, Langley and Anderson raise the following objections: (1) In some experiments in which anatomical connections could be demonstrated they were unable to get any reflex response by stimulating the peripheral stump. (2) It is possible that at a certain stage of regeneration the conductivity of the junctional portion is too small to give a reflex effect under the conditions of the experiment. (3) When the regenerated axons come from the femoral or obturator, as is sometimes the case, stimulation of the central end of the sciatic would not affect them.

Lugaro ('05) saw that the operative methods employed by Bethe, did not exclude the possibility of downgrowth of axons from fibers still connected with the central nervous system. In order to remove all possible sources of such contamination, he resected the lumbo-sacral nerves together with their associated spinal ganglia at their exit through the dura mater in young dogs and cats, and was unable to find any medullated fibers in the sciatic four months after the operation. In three young dogs, in which he removed the lumbo-sacral spinal cord and its associated spinal ganglia he found after three months the same absence of medullated fibers in the sciatic nerve.

Raimann ('05) cut away that portion of the lumbo-sacral spinal cord and associated ganglia from which the sciatic nerve arises; but his positive results, taken in connection with the negative ones of Lugaro, can only show the great tendency for fibers to grow into the degenerated sciatic from the obturator and femoral, which with their associated portion of the spinal cord remained intact in Raimann's experiment.

Segale ('03) is of the same opinion as Lugaro and distinguishes between compensation due to ingrowth from other nerves and

regeneration—processes which are associated in the functional restoration of divided nerves.

We must still mention briefly a few of the less important contributions on the side of autogenous regeneration. Van Gehuchten ('04) repeated Bethe's experiments with positive results, but did not make a microscopic examination of the intervening scar nor attempt to cause the degeneration of the new fibers by secondary section of all the nerves going to the leg. Ballance and Stewart ('01), Fleming ('02), Durante ('04), and Modena ('05), have made observation in favor of an autogenous regeneration of nerve fibers; but since they present no new evidence we need not go into their work in detail here.

Bethe ('07) has attempted to meet the objections raised by his opponents, but, as it seems to the present writer, with very little success. So far as his paper is not a mere repetition of former experiments it may be divided into three parts: (1) He considers the facts brought to light by Cajal's new silver method, but we can best take up Bethe's objections to this method in another part of this paper. (2) He reports experiments to show that when an axon is divided near its cell of origin no regeneration occurs. That his negative results, so far at least as the spinal ganglion is concerned, are to be explained on the basis of inadequate histological methods, will be shown in another paper when the effects of cutting the nerve roots are taken up. (3) He presents experiments designed to meet the objections of Langley and Anderson and Lugaro. In spite of the length already reached by this review it will be necessary for us to analyze these experiments in some detail.

In reply to Lugaro he presents a case, in which after extirpation of all the roots belonging to the nerves going to the hind leg, regeneration was recognizable in the peripheral sciatic stump. In a young dog he tore out both sciatic nerves, but failed to bring the spinal ganglia with them in either case. Several weeks later he opened the canal and removed on the left side the 2 to 7 L. and 1 S. roots. Some months later regeneration could be demonstrated in the peripheral stump. But there is one

serious objection to this experiment, since previous to the removal of the roots and ganglia on the left side he had torn out both sciatic nerves. Hence there was present in the pelvis the torn central fragments of the right sciatic nerve. So great was the regenerative energy of this right sciatic stump that it was found at autopsy that its fibers had grown out of the right great sacro-sciatic foramen and established an anatomically demonstrable connection with the right peripheral stump. Under these circumstances it is hard to convince oneself that a few regenerated fibers may not have taken a different course, grown across the anterior surface of the sacrum out of the left great sacro-sciatic foramen and down to the left peripheral stump. Bethe's other experiments with removal of the roots were negative, a fact which he can only explain as due to the poor state of the general health of the dogs upon which so mutilating an operation had been performed.

To meet the objections of Langley and Anderson he performed two experiments in which several months after the tearing out of the sciatic he tried to cut all possible connections with the spinal cord and six days after the secondary operation still found undegenerated medullated fibers in the peripheral stump. But in one of these cases it is clear that all possible central connections were not divided. In this case the second operation consisted in cutting the roots associated with the nerves going to the leg, and as autopsy showed, he failed to cut the 1 S. root. In the other experiment the secondary operation consisted in (1) cutting the femoral and obturator near the pelvis, (2) cutting the 1, 2, and 3 S. nerves at their exit from the intervertebral canal, and (3) ligature and division of the tissue at the normal point of exit of the sciatic nerve. Since in the primary operation the 6th and 7th L. roots and ganglia had been torn out with the sciatic, this control seems to have been fairly complete. It is plain however that this experiment was very complicated and gave opportunities for error in the operations and subsequent observations. In the opinion of the present writer the positive results of Bethe in these three cases are to be attributed to his habit of tearing out the sciatic nerve, producing a lesion

of unknown extent at an unknown level and rendering difficult the accurate application of Langley and Anderson's test of the presence of central connections by subsequent division of all nerves going to the leg. Positive results obtained by complicated and uncertain methods cannot outweigh the negative results obtained by simpler operative procedures.

More recently Wilson ('09) has repeated some of Bethe's work but came to no definite conclusions as to the nature of the processes involved in the regeneration of nerves.

Space has not permitted a detailed report of all the work that has been done with the object of studying the changes which occur in a piece of nerve permanently cut off from the central nervous system; but enough has been given to show that this line of experimentation has, as yet, given no satisfactory evidence of the capacity of a stretch of nerve completely separated from the central nervous system to undergo an autogenous regeneration. On the contrary, the more recent experiments of this sort show that so great is the regenerative capacity of the central stump in young animals that it is a matter of the greatest difficulty to exclude the possibility of axons from the central having reached the peripheral stump, and it is equally difficult to exclude the possibility of axons having grown into the peripheral stump from other nerves in the vicinity. The only experiments that are entirely satisfactory are those of Lugaro, involving the removal of the entire lumbo-sacral portion of the spinal cord and the associated spinal ganglia, and these experiments were negative.

The reason that so many have attempted to solve the problem in this way is that no stain was available prior to 1903 which was capable of unraveling the complicated processes going on at the point of union of the cut ends of a divided nerve; and any attempt to determine what was going on in this zone with the use of any of the usual stains could scarcely lead to convincing results. Under these circumstances it was but natural that an effort should be made to determine what happened under much simpler conditions in an isolated peripheral stump, not with the idea that any regeneration occurring here would

be essentially different from that occurring when the two ends are in apposition, but with the idea that the process would be easier to analyze, and that in this way evidence would be obtained which could be applied in the analysis of the more complex conditions obtaining in the divided and reunited nerve.

Fortunately we now have in the method of Cajal a technique giving a sharp stain of the finest branchings of axons and enabling the investigator to see clearly the complicated changes going on near the cut surfaces and to correctly understand the mechanism of nerve regeneration. With the solution of the problem, which is given by this new method of staining, there ceases to be any reason for an attempt to secure an isolated stump for study, since it would be highly improbable that there would occur in an isolated stump a process which was essentially different from that which occurs in the peripheral stump when in connection with the central nervous system.

A number of observers have made use of Cajal's method for the study of degenerative and regenerative changes in nerve fibers. Perroncito ('05) and Cajal ('08) have obtained brilliant results. Marinesco ('05, '06), Poscharissky ('07) and Tello ('07) have also made use of this method. As we proceed with the discussion of our own results we will point out in what ways our results differ from those of others who have used this method.

TECHNIQUE

The experiments were made on the sciatic nerve of adult dogs. With strict aseptic precautions the nerve was exposed in the upper part of the thigh and cut with a sharp scalpel or scissors. The ends were in some cases allowed to retract; in others a stretch of 1 cm. was removed and retraction allowed; and in still others, without resection of any of the nerve, the two ends were approximated with sterile silk or catgut sutures. The dogs were allowed to live for a period of from one to thirty-five days and at the autopsy a short stretch of the proximal stump and a longer stretch of the distal stump together with the intervening scar were removed and subjected to histological analysis. Since we deal only with the early stages of regenera-

tion, and with them only from the histological standpoint, little if any information would have been secured by electrical stimulation of the stumps. The omission of these physiological tests of regeneration was the more excusable, since, as has been mentioned, the majority of the autopsies were complete before it was determined to extend the scope of the study from the degenerative changes to include the regenerative processes as well. With two exceptions the wounds healed by primary intention. In Dog III the cutaneous stitches came out on the fourth day, but this dog was killed on that same day and the deeper parts

TABLE 1

NUMBER OF EXPERIMENT	DAYS BETWEEN OPERATION AND AUTOPSY	AMOUNT OF NERVE REMOVED	WHETHER SUTURED OR ALLOWED TO RETRACT
I	1	None	Sutured
II	35	None	Sutured
III	4	None	Sutured
IV	8	None	Sutured
V	14	None	Sutured
VI	19	None	Sutured
VII	34	1 cm.	Retracted
VIII	34	None	Sutured
IX	25	None	Sutured
X	25	1 cm.	Retracted
XI	1	None	Retracted
XII	2	None	Retracted
XIII	3	None	Retracted
XIV	4	None	Retracted

of the wound were free from infection. Dog IX became infected and was discarded. Dog I died about twenty-four hours after the operation probably from late effects of the anaesthetic. The other dogs remained in excellent health until they were killed. Table 1 shows what experiments were made.

The tissue was prepared by the pyridine-silver modification of Cajal's method, the steps of which are as follows: The nerve is placed for forty-eight hours in absolute alcohol to which has been added 1 per cent of strong ammonia. The pieces are then washed for two minutes in distilled water and transferred to pyridine for twenty-four hours, after which they are washed in many changes of distilled water for twenty-four hours. They

are then placed in the dark for three days at 35°C. in a 2 per cent aqueous solution of silver nitrate, then rinsed in distilled water and placed for one or two days in a 4 per cent solution of pyrogallic acid in 5 per cent formalin. Sections are made in paraffin and after mounting are ready for examination. This modification has many advantages over the original Cajal method when applied to the peripheral nervous system. It is only occasionally that one obtains with the older method a satisfactory stain of the non-medullated fibers, while when pyridine is used these fibers stand out with great clearness, not only in their normal state but also in the early stages of degeneration. The various regenerative phenomena in these fibers also stand out with great clearness. Cajal was able with his method to observe some of these changes in the non-medullated fibers but seems to have seen relatively few of these fibers showing that his method was not especially suited for their study. Perroncito ('09), who used the original method, states that "the first phenomenon which is noticed in the peripheral stump of a divided nerve is a clearer differentiation of bundles of non-medullated fibers which run between the medullated fibers." The old Cajal method in the hands of the writer has, only rarely, given a clear differentiation of the non-medullated fibers, and one gathers from the statements of Perroncito and Cajal that it has only been in injured nerves that they have seen them clearly, and even then with less distinctness than that with which they appear in the pyridine-silver preparations. They consider that these fibers are of sympathetic origin and fail entirely to appreciate their great number. As a result of the inadequacy of the old Cajal method many of the most important steps in the degeneration and regeneration of these fibers also escaped their attention. It is evident, however, from their figures and descriptions that when they use the term 'non-medullated fibers' they refer to the same structures which are designated by that name in the present paper.

Another objection to the old Cajal method, as applied to the study of regenerating nerves, is that by this method it is difficult to get both the old and the new axons to stain in the same

preparation, as each requires a different amount of ammonia in the fixing solution (Cajal '08). Cajal advises the use of a mixture of medium strength in order to stain both, but says that in practice it is very difficult to get just the right concentration. All this difficulty is avoided by the use of pyridine, which causes the old medullated axons to stain yellow and the old non-medullated axons, as well as all newly formed fibers, a dark brown or black. The new technique is also much more certain in its results. With pure chemicals and clean glassware no failures need be anticipated. It also has the advantage of giving a uniform stain throughout a large piece of tissue.

Against the use of the Cajal method in the study of regenerating nerves, Bethe ('07) has raised the following objections: (1) The myelin sheaths are not stained. This is of course true and, since these sheaths are represented only by colorless spaces, the method is of little or no value in the study of their degeneration and regeneration. If, however, we wish to study the changes in the axons, this very transparency of the myelin sheaths renders the preparations more serviceable. The very faint yellow of the connective tissue and the transparency of the myelin sheath make it possible to use relatively thick preparations (12 to 15μ) in which the axons can be followed for a relatively long distance. (2) It shrinks the axons. This is also true and is a defect which it has not been possible to overcome. (3) It is uncertain in its action. Bethe excuses himself for not having used Cajal's method by saying that his material was too valuable to jeopardize by the use of such an uncertain technique. This objection certainly cannot be applied to the pyridine-silver method. (4) Only very small pieces of tissue 2 or 3 mm. thick can be used. On the contrary, either with the old Cajal method or the pyridine-silver modification, the best results are obtained with larger pieces. It is, of course, essential that one be able to study large sections in order to secure a correct idea of what is going on at different levels. The preparations which form the basis of this paper are, for the most part, longitudinal serial sections of pieces of nerve from 5 to 8 mm. thick and from 15 to 20 mm. long. They represent sometimes

the central or peripheral stump by itself, sometimes the two united stumps with the intervening scar. These large pieces of tissue were none too large, as the staining is perfect throughout. The use of such serial sections makes it possible to trace with certainty the course of the axons, but has involved the preparation of several thousand sections, and has precluded the use of other stains.

The results of this investigation can best be assembled under the following headings:

Early changes in the distal stump
1. Degeneration of the medullated fibers and formation of nucleated protoplasmic bands
2. Degeneration of the non-medullated fibers and the formation of nucleated protoplasmic bands
3. Abortive autogenous regeneration in the distal stump

Early changes in the proximal stump
1. Changes in the non-medullated fibers
    - Early abortive regeneration
    - Cellulipetal degeneration
    - Formation of new axons
2. Changes in the medullated fibers
    - Formation of a zone of reaction
    - Fibrillar dissociation
    - Early branching of the axons in the immediate neighborhood of the lesion
    - Formation of lateral branches at some distance above the lesion
    - Formation of fiber bundles and skeins

Mechanism of the regeneration of nerve fibers
1. Proliferation of axons in the central stump
2. Penetration of the new axons through the scar
3. Utilization of the protoplasmic bands as pathways for the new axons in the distal stump

EARLY CHANGES IN THE DISTAL STUMP

1. Degeneration of the medullated fibers and the formation of nucleated protoplasmic bands

Leaving out of account, for the moment, the interesting metamorphoses of the nerve fibers which occur in the distal stump in the immediate neighborhood of the cut surface during the first three days, we confine ourselves in this section to the changes

which occur at a distance of at least 5 mm. from the end of the stump. At that distance from the cut surface no changes can be detected in the medullated fibers in Dog XI, killed one day after the operation. The medullated axons still show their characteristic, smooth contour and uniform light yellow stain characteristic of them in their normal state. In Dog XII, killed two days after the operation, many axons have an irregular surface and are stained more intensely but less uniformly than normal axons (fig. 1). The same changes are seen in Dog I, which died about twenty-four hours after the operation. On the third day, although these changes are somewhat more advanced, there is as yet no fragmentation of the axons. This begins on the fourth day and is shown in figure 2. One of the fibers, *a*, is broken up into large granules, staining dark brown and grouped into clumps of irregular size and shape. These masses are for the most part still connected with each other. There is a very marked difference in susceptibility of the axons to degenerative changes. Many of the fibers at this stage are normal in appearance or are in the stage described as characteristic for the second day. Such a fiber, showing only very slight alterations, is seen in figure 2, *b*.

Changes in the myelin sheaths cannot be clearly seen in these specimens. Howell and Huber ('92) found the first evidences of segmentation of the myelin sheath on the fourth day. It is therefore plain that granular degeneration of the axons is well advanced before the segmentation of the myelin sheath begins. This agrees with the observations of Bethe ('03).

After eight days the first 3 mm. of the distal stump are almost completely degenerated; the degeneration becomes less and less marked during the next 2 mm.; and at a distance of 5 mm. from the cut surface we have a condition which is characteristic for the remainder of the distal stretch included in the section (an additional 5 mm.). It is probable that this stage of degeneration would be found throughout all the rest of the distal portion of the nerve. It is also probable that those who have described the degeneration as progressing from the point of injury downward along the nerve have been misled by this rapid

degeneration in the first few millimeters of the stump. All measurements of this sort, referred to in this paper, were made on longitudinal sections with the aid of the microscope and mechanical stage.

At a distance of 5 mm. from the cut surface eight days after the operation, the axons of the medullated fibers are broken up into small irregular clumps of dark brown granules (fig. 3, *a*). The myelin sheaths are divided into elliptical segments, separated and surrounded by the now abundant protoplasm of the neurilemma cells, and containing the granular remains of the axons. In the preparations of this stage the nuclei are not differentiated, although it is known from the observations of others that at this time the nuclei of the neurilemma are rapidly increasing in numbers. There are a few medullated fibers whose axons are not fragmented, but are stained very intensely, have a fairly uniform contour and an intact myelin sheath. Cajal ('08) has called attention to these resistant fibers. Since there are all gradations between the most susceptible and the most resistant, there seems to be involved not a distinction in the kind of fiber, but in the functional state, nutritive condition, and vitality of the fiber at the moment of the lesion.

Fourteen days after the operation there are no longer any unfragmented medullated fibers, although some are in the early stages of degeneration. In most of the fibers the protoplasm has increased in quantity and the myelin is divided into droplets, while the remains of the axons are less in evidence. Nineteen days after the operation the protoplasmic bands of Büngner have made their appearance (fig. 4). The small quantity of protoplasm surrounding the nuclei in the normal fiber has increased and surrounded the fragments of myelin. And as the remains of the axon and myelin have been absorbed, this protoplasm has come to fill the old neurilemma sheath. In the meantime the nuclei have increased in number. As a result, we have a continuous band of protoplasm containing many nuclei (fig. 4, *a*), and occasional droplets of myelin (*b*). Scattered through the remains of the myelin are fine, darkly staining granules representing the remains of the axon. The protoplasm some-

times appears distinctly striated, as is shown in the drawing at one end of the band. Such a longitudinal striation has lead Büngner, Bethe, and others to suppose that they had before them the beginnings of a new axon. Even Marinesco ('05) using Cajal's method was at first led into this error; but later, perhaps with better preparations, he changed his view and adopted the conception of the outgrowth of the axon from the central stump. There are absolutely no transition stages between such striations and the new axons, which, when they first appear in the peripheral stump, are sharply defined from all surrounding structures (fig. 28). Even at this stage, nineteen days after the operation, these protoplasmic bands are capable of serving as paths for the developing axons. A considerable number of fine black fibers can be seen in the proximal part of this distal stump. They lie in the protoplasmic bands and run around the droplets of myelin. These new axons can be followed by a study of the serial sections back to the scar through which they have traveled from the central stump.

In Dog x, in which 1 cm. of the nerve was excised, a careful study of serial sections fails to show any axons which have pierced the intervening scar and entered the distal stump. In these preparations, therefore, we are able to study the protoplasmic bands without any complication from ingrowing axons. The length of time from operation to autopsy in this experiment was twenty-five days. Most of the detritus from the axons and myelin sheaths has been absorbed, but one sees scattered through the field a few good sized droplets. The protoplasmic bands have assumed a fairly uniform contour and stain a light yellow. Fine black granules are scattered through the protoplasm. These are sometimes arranged in parallel rows, giving a striated appearance. In none of these fibers is there any indication of the beginning of an axon. This is significant when taken in connection with the preceding case in which the two stumps were united, and in which, although the time between operation and autopsy was six days shorter, new axons could be seen in many of the protoplasmic bands in the neighborhood of the scar.

In the dog which was killed thirty-four days after the removal of 1 cm. of the sciatic nerve, the distal stump showed protoplasmic bands like those already described except that the droplets of myelin were smaller and scarcer. A few showed new axons within them; but these axons could be followed for long distances, were sharply differentiated from the protoplasm of the band and were connected with fibers in the scar. No transition stages could be seen which might be interpreted as the development of axons in situ. More will be said about the relation of the new axons to the protoplasmic bands in the last section of this paper.

2. Degeneration of the non-medullated fibers and formation of nucleated protoplasmic bands

So far as we are aware no one has described the degeneration of the non-medullated fibers of the spinal nerves. Tuckett ('96) presents experiments showing that the sympathetic axons lose their affinity for methylene blue on the second day after separation from their cells of origin. Cajal and others who have used the silver method as he directs have apparently been able to see these fibers in the spinal nerves only when they were undergoing regenerative or degenerative changes and possessed an increased affinity for the silver. Cajal states that he has followed the degeneration of the non-medullated fibers during the first eight days, but his observations clearly refer only to the immediate vicinity of the lesion where the fibers have undergone an abortive regeneration. He states that the centrally directed end bulbs (which we will describe in another place) and the non-medullated fibers which carry them, stain well until the third day, after which they gradually fade out and are no longer visible after the sixth or seventh day. These statements are correct only when they are made to apply to the distal stump within 1 mm. of the cut surface.

At a distance of 5 mm. from the cut surface one day after the operation (Dog XI), many of the non-medullated fibers are no longer uniformly stained but are distinctly granular. At the end of the second day (Dog XII), the fibers become broken

up into segments of varying length. Short, light segments alternate with longer, very much darker segments (fig. 5). The dark segments are granular, a detail which has been omitted in the drawing. For control we have the normal nerve from Dog XI, carried through the same solutions at the same time as the divided nerves of Dogs XI and XII. As these changes are not seen in the normal nerve, we may be sure we are not dealing with artifacts. Three days after the operation, the darker segments, still granular in appearance, stain less intensely than in the specimen taken a day earlier. A considerable number of smooth, black, uniformly stained, non-medullated fibers can be seen—but these are not so much in evidence as at a later date when the other fibers have undergone more complete degeneration. Again in this experiment we have the normal nerve carried through the same solutions at the same time as the divided nerve, to serve as a control, and show that the described changes are not artifacts due to an irregular deposit of silver.

Two dogs were killed four days after the operation. Both showed only a little advance over the specimen taken on the third day; but many of the fibers are already taking a light yellow stain with little differentiation into lighter and darker segments.

After eight days many of the non-medullated fibers are no longer visible, others appear as light yellow bands along which an occasional nucleus can be seen.

It is at this stage when the other fibers are very lightly stained or have disappeared that the resistant undegenerated fibers are most evident. These are more numerous and persist longer than the resistant medullated fibers. Since the majority of the non-medullated fibers degenerate very early and since there are few fibers which show an intermediate degree of resistance, it is possible that we are dealing here with two different kinds of fibers. Those which degenerate early in the first week may be afferent spinal fibers; while those which degenerate in the second and third weeks may be of sympathetic origin. This supposition is the more probable because the susceptible fibers represent about the same proportion, which on other grounds

we would assign to the class of afferent spinal non-medullated fibers, while the resistant fibers correspond in number to the fibers of sympathetic origin. Cajal ('08) in one place called attention to the slow degeneration of the non-medullated fibers, overlooking entirely the much larger number which degenerate very early, while in another place in the same monograph he states that all the non-medullated fibers have disappeared by the sixth or seventh day.

In Dog v, fourteen days after the operation, the non-medullated fibers are represented by narrow yellow bands stippled with dark brown fine granules. The nuclei are not differentiated in this preparation. A considerable number of resistant non-medullated fibers still retain their intense black uniform stain but they present for the most part an irregular contour (fig. 6). Some of these fibers seem to be present after nineteen days, but in this specimen new axons have grown in from the central stump and might be confused with persistent axons. The peripheral stump of the specimen taken twenty-five days after the operation is not contaminated with new axons from the central stump and here it can be seen that all the old axons have degenerated.

In Dog v, nineteen days after the operation, the degenerated non-medullated fibers are more clearly visible than on the eighth and fourteenth day, and the nuclei upon them are well differentiated. The fibers are fine yellow bands with many nuclei. Surrounding many of the nuclei there are considerable accumulations of protoplasm. A short section of three such fibers is represented in figure 7. The fibers are not interrupted as the drawing would indicate, but can be followed for considerable distances even in relatively thin sections. The fibers and the protoplasm about the nuclei still contain dark brown granules. After twenty-five days these fibers present the same picture as in the preceding specimen. They are clearly differentiated and the nuclei are sharply stained. These fibers are grouped in bundles and lie between the protoplasmic bands formed by the medullated fibers, from which they can be distinguished by their small size and by the absence of myelin droplets.

In the preceding paragraphs we have recorded the changes as they can be observed from day to day in the degenerating non-medullated fibers. These observations may be summarized and interpreted as follows. The axons of the majority of the non-medullated fibers begin to degenerate within twenty-four hours after they have been separated from their cells of origin. They first become granular and after two or three days become broken up into segments of lighter and darker staining. The darker stained segments represent the fragmented axon, the lighter segments represent accumulations of unstainable substance probably of fluid character. It is not easy to determine whether the active process is a vacuole formation with extension in the long axis of the fiber causing separation of the fragments of the axon, or whether a retraction of the fragments leaves the empty spaces within the neurilemma to be filled with exudate. On the fourth day the dark segments have begun to disintegrate and by the eighth day the dark segments have disappeared, the remains of the degenerated axon are distributed as brown granules through the probably fluid contents of the neurilemma sheath. During the next stage, eight to fourteen days after the operation, the fibers are not very clearly seen. But by the nineteenth day the nuclei of the neurilemma have increased greatly in number and the fibers again become clearly visible, since with the increase in the number of nuclei the protoplasm has also increased and filled in the old neurilemma sheath. We have, therefore, as the terminal stage of the degeneration of the non-medullated fibers nucleated protoplasmic bands which differ from the similar bands formed from the medullated fibers only in size and in the absence of myelin droplets.

In this connection certain observations of Perroncito ('09) are of great interest. He noticed that in the neighborhood of the bundles of non-medullated fibers spindle shaped cells appeared on the third and fourth days and that these became more numerous on the seventh and eighth days and lay in connection with bundles of connective tissue fibers. He failed to recognize the formation of the protoplasmic bands from the medullated fibers and stated that at last "in place of the nerve

there remains a connective tissue strand consisting of connective tissue fibers and very long spindle cells." These cells, according to him, arise from the spindle shaped connective tissue cells which appear between the nerve fibers shortly after division of the nerve. He adds:

> But before we assume that these are true connective tissue cells, we must seriously consider one objection. We know that between the medullated nerve fibers there were bundles of non-medullated fibers. Could not these spindle cells represent cells associated with the non-medullated fibers and be homologous to the cells of Schwann's sheath? This objection has, however, many weak points.

It is clear that Perroncito is here describing the same phenomena which we have interpreted as the formation of protoplasmic bands from the non-medullated fibers. His drawings show, however, that his preparations clearly differentiated little more than the nuclei in question. The pyridine-silver preparations, on the other hand, clearly differentiate, not only the degenerating non-medullated fibers, but also the protoplasmic bands derived from them.

While a few non-medullated fibers, probably of sympathetic origin, may persist for two or three weeks, all non-medullated fibers in the peripheral stump undergo degeneration before the end of the fourth week. This shows that the direction of Wallerian degeneration is the same for the non-medullated as for the medullated fibers, and excludes the possibility that any of them might arise from cells located at the periphery, unless one cares to consider the very remote possibility that the slowly degenerating non-medullated axons undergo a cellulipetal degeneration toward peripherally located sympathetic ganglion cells.

3. Abortive autogenous regeneration in the distal stump

It will be remembered that all the descriptions and drawings in the two preceding divisions of this paper refer to the distal stump at a distance of at least 5 mm. from the cut surface. It was necessary to choose a point at some distance from the cut surface, because changes occur in the immediate neighborhood of the lesion which are essentially different from those seen in

the remainder of the peripheral stretch. These changes have been described by Perroncito ('05), Poscharissky ('07), and Cajal ('08), with whose observations our own are in full accord.

These changes begin very early. One day after operation they are already well adanced in both the medullated and non-medullated fibers. In the millimeter nearest the cut surface the non-medullated fibers possess lateral branches, and the fibers as well as their branches end in bulbs (figs. 8 and 9). The mode of formation of these branches is the same as the mode of formation of the similar branches seen in the immediate neighborhood of the lesion in the proximal stump; but, since the successive stages are more clearly seen there, we will describe their formation when describing the central stump. The side branches are usually as large as the parent stem. The end bulbs are very characteristic, differing markedly from those seen on the ends of fibers taking part in the ultimate regenerative changes. These bulbs have a darkly staining core with a neurofibrillar network, and a peripheral lightly staining zone, which is often of considerable thickness and shows no visible neurofibrils. These side branches are so numerous and their end bulbs so large that they separate widely the original fibers of a bundle. The last 0.5 mm. of the stump assumes a characteristic appearance under low magnification due to the replacement of the parallel bundles by a network, the individual fibers of which are separated by large end bulbs. It seems probable that the outer lightly staining zone of these end bulbs represents an excessive accumulation of interfibrillar substance.

The growth of lateral branches and the formation of end bulbs does not progress much, if any, after the first day, but remains stationary during the second, third and fourth days. We have unfortunately no intermediate stages between the fourth and eighth days, and during this time the products of this abortive regeneration have almost entirely disappeared. There are to be seen after eight days shadowy outlines of the bulbs and a somewhat more definite indication of the branched fibers. A few of the fibers and end bulbs are still clearly stained. After fourteen days there are still a few scattered fibers show-

ing end bulbs and one small fasciculus at the periphery of the nerve in which many well stained side branches and bulbs are seen. These resistant elements correspond to the central ends of the resistant non-medullated fibers, which, it will be remembered, retained their staining reaction for a period of two or three weeks. All of these resistant fibers with the branches and bulbs at their central ends have entirely disappeared before the twenty-fifth day and no traces of them are to be found in the preparation taken on that day.

Changes of the same nature occur in the medullated fibers. In the immediate vicinity of the lesion (0.5 mm.) many of the medullated axons present a zone of reaction, which separates the, as yet, normally staining distal stretch, from a very short disintegrated piece of the axon at the cut surface. This zone of reaction becomes more marked on the second and third days. There is almost no limit to the variety of appearances which the reaction zone may assume; but the fiber illustrated in figure 10 may be taken as showing the principal factors involved. The myelin sheath in this part of the fiber is already broken up and appears as granular detritus filling the old sheath. Imbedded in this mass we have the axon and its products. The central and peripheral ends of the fiber are marked *c* and *p*, respectively. The proximal part of the axon has completely degenerated; fragments of it can be seen at *a*. At *b*, the axon shows a sharply defined club-shaped enlargement which represents the end of the living part of the axon. Following the axon in a peripheral direction, one sees at the distal end of the enlargement a distinct neurofibrillar reticulum. Below this the axon contracts, expands, contracts again and goes over into a second wider meshed fibrillar reticulum. From both of these reticula fine black fibrils, *d*, are given off which run by themselves in the degenerated myelin. These isolated fibrillar branches are also seen on the central side of the club-shaped enlargement. The reaction in the medullated axons therefore consists of (1) the formation of a distinct line of separation between a short dead segment and the remaining distal stretch of the fiber, which is still living, (2) the appearance near this line of separa-

tion of a distinct neurofibrillar reticulum, and (3) the formation of fine fibrils which leave this reticulum and run independently through the degenerated myelin.

The manner of formation of at least some of these independent fibrils is shown in figure 11. In the peripheral part of the drawing (*p*) there is a well-marked neurofibrillar reticulum from one side of which there arises a lateral branch ending in a bulb showing a distinct reticulum. On the central side this fiber presents two branches *c* and *c'*. It is probable that *c'* represents a pre-existing collateral and not a new formed branch. Most of the fibrils arising from the central end of the reticulum pass into this branch.

Many, perhaps a majority, of the medullated axons, never show any of these changes but undergo an uncomplicated degeneration throughout their entire extent. Those fibers which show no reaction near the lesion represent, in all probability, the more susceptible fibers, which are well advanced in degeneration throughout their entire extent by the fourth day. The more resistant the fiber, the more marked is the reaction near the lesion and the more tardy is the fragmentation of the remainder of the distal stretch. By the eighth day most of the products of this reaction have disappeared and by the fourteenth day there are no longer any traces of them.

The changes which have been described in the medullated and non-medullated axons of the distal stump are without significance for the ultimate regeneration of the nerve, since all the products of this reaction suffer complete degeneration and disappear. In themselves, however, these changes are of the highest interest. They show that that portion of a fiber which is separated from its trophic center does not die at once. It continues to live for two or three days and possesses sufficient vitality to cause a rearrangement of its fibrils into a complicated reticulum and to give rise to lateral branches. The presence in reacting medullated fibers of fine fibers budding off from the side of a reticulum as shown in figure 11, and the fact that some of these fine fibers pierce the neurilemma and run out into the endoneurium can only be interpreted as true branching, although

it is probable that others of the independent fibrils within the old sheath have gained their independence by the degeneration of the fibrils which surrounded them. In the case of the non-medullated fibers the lateral branches with their end bulbs constitute the chief evidence of the reaction. One might assume that the appearance of the neurofibrillar reticulum was only a peculiar manifestation of degeneration. But the formation of new branches can only be interpreted as regenerative in character and accepted as evidence that the axons live in the distal stump for some time after being severed from their trophic centers. Cajal ('08) places the same interpretation on these phenomena.

Harrison ('08) found that, after cutting the nerves of the tail of the larvae of Rana sylvatica, the two cut ends of many nerve fibers had united by a protoplasmic bridge within one or two days. In these fibers the degeneration of the peripheral part of the axon was immediately arrested. These observations find support in the evidence just presented to show that a portion of an axon severed from its trophic center continues to live for two or three days.

While Cajal ('08) and others, who have used his method in the study of the regeneration of nerves, saw and described the changes in the non-medullated fibers of the distal stump, just as they have been described here, they leave the impression that only a few scattering fibers are involved. They seem, also, to have overlooked the fact that exactly similar changes occur in the non-medullated fibers of the proximal stump.

EARLY CHANGES IN THE PROXIMAL STUMP

1. Changes in the non-medullated fibers

The changes in the non-medullated fibers may be divided into three stages: (a) early abortive regeneration; (b) cellulipetal degeneration; (c) the formation of new axons.

a. Early abortive regeneration. By early abortive regeneration in the non-medullated fibers of the proximal stump we mean to designate a reaction exactly analogous to the abortive regen-

eration of these fibers in the distal stump. In the last 0.3 mm. of the proximal stump one sees at the end of twenty-four hours the formation of lateral branches on the non-medullated fibers. The stages in the formation of these branches are illustrated in figure 12. The earliest stage is seen at *d*, and consists in the development on one side of the fiber of a spherical mass several times thicker than the fiber and staining only a trifle less intensely than the fiber itself. These masses seem to possess the power of ameboid movement and work their way out of the fiber bundle in which they orignated, pulling on the fiber and forming a *V*-shaped bend in it. Three stages of this are seen at *d*, *c* and *e*. As the mass moves farther it comes to be connected with the *V*-shaped bend in the old fiber by a single limb which is formed behind the mass as it moves forward. Thus the *V* becomes transformed into a *Y* (fig. 12, *b*). As the lateral branch is formed it often happens that the distal limb of the *V* degenerates. The distal limb of the *V* in *c* can be followed only a short distance when it goes over into a finely granular thread (not shown in the drawing) and disappears. The fiber and bulb seen at *a* are to be accounted for in this way. The bulb appears as if on the end of a fiber which has turned at right angles and left the bundle.

The composition of the bulbs on the ends of the lateral branches varies. At first (fig. 12, *c*, *d*, *e*) the neurofibrillar content is fine and uniformly distributed, giving to the bulb a stain almost as dark as that of the fiber. At this stage the fibrillar plexus can only rarely be seen. As growth continues the interfibrillar substance increases greatly in quantity and out of proportion to the increase in fibrillar substance. This may, in rare cases, lead to the formation of a wide meshed neurofibrillar reticulum (fig. 12, *b*) or more often to the accumulation of a large mass of lightly staining substance about a central fibrillar core. This central core is about the size of the original bud from the side of the fiber.

It should be noted that the bulb shown at *b* is an exceptional one in the clearness with which the fibrillar reticulum is stained and in the fact that it gives off secondary branches. There

were two of these secondary branches, each ending in a little ring. More than one lateral branch may be given off from a single non-medullated fiber. On one fiber two such buds in the early stages of formation were separated by not more than twice their own diameter from each other.

These early branches do not appear to develop much after the first day and on the second day are already overshadowed by the transformations of the axons of the medullated fibers. On the third day, coincident with the beginning of a cellulipetal degeneration in the fibers from which they arose, these branches and end bulbs become indistinct in outline and begin to lose their affinity for the stain. And after four days one can distinguish only indistinct shadowy outlines of the bulbs and fibers (fig. 18, *c*). These products of the early regenerative activity of the non-medullated axons in the proximal stump are therefore abortive and very rapidly undergo degneration. It is an interesting fact that their development in the proximal stump did not progress as far as that of the similar structures in the distal stump and underwent degeneration earlier. This early abortive regeneration is an expression of the local vitality of the cut fiber just as is that of the peripheral stump. The reason for the final degeneration of these products is to be found in the fact that a cellulipetal degeneration occurs in the fibers from which they arose, thus cutting them off from their connection with the cell body.

b. Cellulipetal degeneration. Coincident with the beginning of the degeneration of the products of the non-medullated fibers near the cut surface, changes are noticed in the fibers themselves. These changes, first noticed on the third day, are well advanced on the fourth and extend up the fibers as far as they are included in the section. On the eighth day the alterations in these fibers at various distances from the cut surface (measured with the aid of a mechanical stage) are as follows: In the last 0.5 mm. there are only a few shadowy outlines of the non-medullated fibers; at 3 mm. from the cut surface the fibers can be clearly seen but are in the late stages of degeneration similar to those in the distal stump, on the same day and at the same

distance from the cut surface. From this point to a point at a distance of 10 mm. from the lesion the intensity of the degeneration decreases until at 10 mm. there are exhibited changes similar to those seen at the end of the second day in the distal stump. There are here the same breaking up of the fibers into light and dark segments and the same granular staining as has already been described in the distal stump. In the proximal stump, however, there is the important difference that the intensity of the process decreases rapidly in a central direction; and although our section, only a little more than 1 cm. in length, does not permit us to say at just what point the degeneration ceases, there can be little doubt that it does not extend more than 2 cm. up the nerve. We are dealing here with a cellulipetal or retrograde degeneration.

A slow, ascending, cellulipetal, or retrograde degeneration has been noted in the medullated fibers in cases of long standing amputation (Ranson '06). It is questionable whether such a degeneration occurs in any of the medullated fibers within the time covered by this series of experiments; no clear evidence of its occurrence could be found after thirty-four or thirty-five days. Its occurrence in the non-medullated fibers is of special interest since it indicates a greater susceptibility of the neurones, of which they form a part, to lesions of the peripheral nerve. On cutting the dorsal ramus of the second cervical nerve in the white rat, near the ganglion, Ranson ('06) noted that 52 per cent of the cells in the associated ganglion underwent complete degeneration. It was later shown (Ranson '09) that the cells which disappeared were the small cells, which we now know to be associated with the non-medullated fibers; while very few, if any, of the large cells, which are associated with the medullated fibers, disappeared.

While in those experiments the cut was made very close to the ganglion, in the present series of experiments the sciatic was divided at a relatively great distance from the ganglion. And, following the law that the intensity of the reaction in the cell depends upon the proximity of the lesion in the axon to the cell-body, the division of the non-medullated fibers

in the sciatic involved only a limited cellulipetal degeneration. While, of course, the usual axonal reaction must have occurred in the small cells, these did not degenerate. The spinal ganglia associated with the injured sciatic nerve in Dogs VII and VIII, killed thirty-four days after the operation, and Dogs IX and X, killed twenty-five days after the operation, were prepared by the pyridine-silver method. In these ganglia the small cells and their associated non-medullated fibers were apparently as numerous as in normal ganglia, although, since no counts were made, it would be impossible to be sure that none had degenerated. The non-medullated fibers in the ganglia and adjacent portion of the nerves stained in a perfectly normal manner, showing that they were not involved at that level in a cellulipetal degeneration. Another piece of evidence, showing that the cellulipetal degeneration in the neighborhood of the lesion did not involve the degeneration of the entire neurone, is found in the subsequent regeneration of these fibers.

c. Formation of new axons. On the fourteenth day the majority of the non-medullated fibers in the proximal stump are still in the late stages of degeneration. They appear as light yellow, delicate bands, closely resembling the early stages of protoplasmic band formation in the distal stump. There are present, however, on the fourteenth day, many sharply stained black fibers in the bundles of light yellow ones. One bundle, in which the regenerative changes have gone particularly far, is seen in figure 13. In a bundle of sharply staining fibers one sees five end bulbs, three directed toward the periphery, *p*, and two towards the center, *c*. From this time on there is a constantly increasing number of sharply staining black fibers in these bundles. On the nineteenth and twenty-fifth days there are numerous end bulbs and an increasing number of fine black fibers. After thirty-four days the bundles of non-medullated fibers in the last centimeter of the proximal stump are larger and more compact than in the normal nerve; and these bundles can be traced, in longitudinal sections, out of the cut end of the nerve into the scar. A cross section of the stump taken a short distance above the cut at this time shows a great increase in the number of these fibers (fig. 26).

The interpretation which is to be placed on these observations is as follows: The non-medullated fibers degenerate a short distance (about 2 cm.) in a central direction and the degenerated portions undergo the changes looking toward the formation of protoplasmic bands. But before these are fully formed the cellulipetal degeneration has been arrested and regeneration begins. The fibers above the point where the degeneration ceases begin to grow downward and on their ends bulbed extremities can be seen. The great increase in the number of these fibers in the proximal stump near the lesion indicates that there also are formed lateral branches, although on account of the compactness of the bundles it has not been possible to observe the branching directly. Cajal describes and figures the branching of non-medullated fibers in the proximal stump, but it seems probable that he was dealing, not with the original non-medullated fibers but with new axons, which had branched off from the medullated fibers (fig. 22). The new non-medullated axons follow the old fiber bundles as pathways and at the cut end of the nerve they finally reach the scar, into which they run.

2. Early changes in the medullated axons of the proximal stump

a. Formation of a zone of reaction. In the immediate neighborhood of the lesion one sees changes in some medullated axons that greatly resemble those seen at an early stage in the distal stump. Figure 14 represents a typical fiber of this sort at the end of the first day. At *d* one sees the axon staining like the normal axon a light yellow; but it is beginning to increase in diameter. As we follow it from the central (*c*) toward the peripheral end (*p*) it increases in size and assumes a darker stain. At the same time, indications of a neurofibrillar reticulum make their appearance. At *b* the axon is several times its normal thickness, filling and distending the neurilemma. It shows at this point, and for a short distance above, a dense deeply staining reticulum. This corresponds to the club-like zone of reaction seen in figure 10, except that the neurofibrillar reticulum is more pronounced. At *a* the disintegrated remains

of the axon can be seen and this degenerated stretch extends to the cut surface 0.2 mm. distant. As can be seen, there is not a very sharp border between the degenerated portion of the axon and the zone of reaction. Isolated portions of the reticulum extend into a lightly stained intermediate zone. At *a*, the neurilemma can be seen; at *b*, it is stretched over the swollen axon and is not differentiated, while at *c*, both the neurilemma and myelin sheath can be seen in the preparation but were not indicated in the drawing. More rarely one sees two dense fibrillar reticula, separated by a zone of light staining through which run a few connecting fibrils.

b. Fibrillar dissociation. In many axons there occurs a separation of the neurofibrils due to accumulation of an excess of interfibrillar substance, and at the same time the individual fibrils become much more sharply stained. This process occurs alike in fibers which have formed a zone of reaction and in those which have not. Figure 15 represents a short stretch of an axon 0.5 mm. from the cut surface two days after the lesion. On following this axon toward the cut surface it is seen to go over into a narrow band of fibrils which connect it with a zone of reaction near the lesion. Between the part of the axon, which was drawn, and the zone of reaction there is a stretch of about 0.2 mm. in which most of the axon is fragmented and only a narrow band of fibrils connects the two. The accumulation of interfibrillar substance seems to be more abundant on one side of the axon where large spaces are present between the fibrils. There does not seem to be any new formation of fibrils and only such rearrangement as would naturally take place in case of an oedematous swelling of the axon. It will be noticed that the network is most dense at the periphery of the axon. When this process is carried to its full extent, as one sometimes sees it on the second, third and fourth days, the axon becomes converted into a fine meshed network forming a hollow cylinder. The myelin sheath having disintegrated, this net-like cylinder lies immediately beneath the neurilemma. These appearances would, of course, be best understood on the hypothesis that the fibrils of the normal axon are not isolated but are united

with each other to form a network, the meshes of which are drawn out in a longitudinal direction.

c. Early branching of axons in the immediate neighborhood of the lesion. The fibers which give rise to these branches undergo no degeneration at the point of division nor do they form at their cut extremity a darkly staining zone of reaction. We are dealing here with fibers which either possessed a greater vitality or were less severely traumatized in the cutting of the nerve. There seem to be two chief modes by which the branches arise from these fibers:

1. Some of these fibers begin to grow distally very soon after the lesion and by the end of the first day have grown out of their sheaths into the exudate. Here they immediately break up into a great number of fine branches. Figure 16 represents two such fibers one day after the operation. At *c* and *c′* they are leaving their sheaths and growing into the exudate. A large fusiform lateral branch is given off from one of the fibers. This large lateral branch, as well as the two main fibers, show the neurofibrils very clearly—these fibrils are however more nearly parallel in their arrangement and not so closely set as in the zone of reaction shown in figure 14, *b*, and on the whole give the ends of the fibers a much more normal appearance. At many points these axons give off fine fibrillar branches which end in small cylindrical or spherical expansions. In the surrounding exudate there are numerous isolated rings which represent cross sections of the expanded ends of other fibers. This is a very characteristic ending for the fine branches of the medullated axons at this stage—an elongated club-shaped end bulb whose fibrillar substance is located at the periphery, in cross section appearing as rings and in longitudinal section as hollow clubs.

2. Instead of arising from the ends of axons, extremely fine side branches may arise from the surface of the axon within its sheath. Axons giving rise to branches of this sort show no degenerated stretch near the lesion nor any dark zone of reaction. They show however a certain amount of fibrillar dissociation in that the axons are swollen and the fibrils are much

more sharply differentiated than in normal axons. Some of these fibrils detach themselves from the axon at its surface to form fine branches, which run in various directions on the surface of the axon and after a short course end in small bulbs (fig. 17). Some of these branches, which arise near the cut surface find their way out into the exudate, but most of them continue to grow within the old sheath. In the absence of the myelin sheath, which is entirely disintegrated in these last few tenths of a millimeter of the proximal stump, these branches reach the neurilemma and immediately underneath this they continue to grow in a circular or spiral direction, interlacing with one another. In this way there develops by the third or fourth day, just beneath the neurilemma, a hollow cylinder formed by fine interlacing fibers, within which the old axon can be seen (fig. 18, *a*, *b*). Many of these fine fibers can be seen ending in little rings. While the majority of them remain within the old neurilemma sheath, a few, leaving it at the cut surface, run into the exudate and a few others, piercing the sheath, run into the eudoneurium.

In figure 19 the axon is seen giving off some relatively coarse branches, some of which have arranged themselves in a tubular sheath. The axon and part of the sheath are cut away in one place, where they run out of the section.

In some of the medullated axons the fibrillar dissociation represented in figure 15 and the branching illustrated in figure 17 seem to be associated in the production of a tubular network. Sometimes the central axon can be seen to break up into this network and be reformed from it again at a lower level.

The formation of a zone of reaction, the fibrillar dissociation, and the formation of the early collateral branches within the old neurilemma sheath, together with the tubular networks which have just been described, constitute the early changes in the medullated axons which were first seen by Perroncito and which Cajal has called Perroncito-phenomena. On the eighth, fourteenth and nineteenth days all of these structures become less and less evident, and their place is taken by parallel coursing fibers, of which there are a large number within

the remains of one old neurilemma sheath. The steps in this substitution are not clearly presented in the preparations studied. But it seems probable that some of the fibers of the network atrophy and that others assume a more parallel course. It is certain that many branches arise from the axons at a slightly higher level and, growing down within the neurilemma sheaths, help to take the place of the disappearing network. It is obvious that these plexuses, as such, take no part in the final regeneration, although individual fibers, derived from them, may do so. Nineteen days after the operation none of the old networks are to be seen; but instead, only parallel coursing fibers, branching fibers and twining fibers held together in bundles by the old neurilemma sheaths. All of these earliest phenomena are confined to the last few tenths of a millimeter of the proximal stump.

d. Formation of lateral branches at some distance above the lesion. On the eighth day, when the tubular arrangement just described has begun to disappear, one sees that the medullated fibers are giving off lateral branches at a higher level. These are of good size and can be seen coming off as high as 5 mm. above the cut. These side branches become more and more abundant in each of the three succeeding stages, fourteen, nineteen, and twenty-five days after the operation. On the nineteenth day, they can be seen in large numbers (fig. 20) chiefly in the terminal 5 mm. of the nerve. They end in bulbs and usually run within the old sheath, predominantly in a peripheral direction, but some run spirally, and others centrally, in the old sheath, and still others pierce the sheath and run in the endoneurium. In figure 21 one sees a bundle of fibers formed in this way within an old sheath. The old axon is thicker, lighter and more centrally placed than its branches which are accompanying it toward the periphery. Two of these branches are seen to end in bulbs directed peripherally. Two fine fibers leave the sheath and run in the endoneurium. One branches a second time in the connective tissue and the resultant fibers run out of the section. The other turns peripherally parallel to and just outside the old sheath and ends in a large end bulb.

After thirty-four days all the medullated fibers in the last 5 mm. of the proximal stump are transformed into bundles of fibers in this way. In Dog VIII, in which the nerve was sutured and in which therefore the chemiotactic influence of the distal stump was brought into full action on the growing fibers of the proximal stump, most of the newly formed axons grow directly downward into the scar. As is shown in figure 23, the fiber bundles are composed of more or less parallel fibers. In Dog VII, however, where a piece of nerve was resected and the influence of the distal stump therefore was less potent in controlling the course of the growth of fibers in the central stump, there are more recurrent fibers. Some fibers instead of passing directly down the old sheath grow in a spiral direction beneath the old neurilemma, producing tangled skeins like that illustrated in figure 24, *b*. The medullated fiber (*a*) has been transformed into a bundle of parallel fibers. These structures are seen in cross section in figure 25. In all these bundles and skeins, bulbs on the ends of individual fibers are seen and form a prominent part of the picture. These two bundles in figure 24 lie side by side in the preparation and a small group of fibers can be seen leaving Bundle *a* and running into Bundle *b*. While therefore on the whole such a bundle represents the branches of one old axon, branches may grow from one bundle into another or from a bundle into the endoneurium. Both recurrent fibers and tangled skeins are also seen in smaller numbers in Dog VIII where union of the cut ends favored the action of the chemiotactic influences from the distal stump on the growing axons.

Fibers with bulbous extremities are very numerous in these bundles and skeins. These end bulbs form a prominent part of the picture in all the preparations where regenerating axons are seen. Cajal has correctly interpreted them as analogous to the enlarged extremities seen by many observers on the ends of developing nerve fibers and hence they may be regarded as the growing tips of the axons.

Cajal believes that the tangled skeins arise from the tubular networks which appear shortly after the lesion. This, however, does not seem to be the case. They arise from the lateral

branches given off from the axons at a later date and at a higher level. When these branches instead of growing in a peripheral direction, coil spirally beneath the neurilemma or turn backward in a central direction, they produce the tangled skeins. These skeins are seen in the process of formation on the twenty-fifth day at a time when all of the tubular networks have disappeared and are often seen farther up the nerve than the level at which the tubular networks appeared. The fact that they are more abundant, when the union of the cut ends is prevented, is readily explained by the less potent attraction exerted on the growing branches by the distal stump, but would not be explained by Cajal's hypothesis.

Cajal is of the opinion that the normal mode of regeneration of the medullated axons consists of the three following stages: (1) degeneration of a short stretch of the axon near the cut surface; (2) the formation of a bulb on the axon above this stretch; and (3) growth of the axon out of the sheath into the scar where branching occurs. This sequence of events occurred in few, if any, of the fibers in our sections. In our preparations the essential factor in the regeneration of the medullated axons of the proximal stump was the formation of lateral branches at a distance of 1 to 5 mm. above the cut surface and within the old sheaths, within which they grew peripherally until they reached the scar. The fact that Cajal worked chiefly with young dogs and rabbits, while this work has been done on adult dogs is sufficient to explain this difference in the results.

The recent statements of Dominici ('11) that no new axons can be observed growing out of the proximal stump during the first month are not supported by an account of detailed observations, and his negative results are clearly due to an inadequate technique.

MECHANISM OF THE REGENERATION OF NERVE FIBERS

We have been concerned in the preceding sections of this paper with a variety of changes occurring in the proximal and distal stumps, many of which, so far as we can see, take no

part in the ultimate regeneration of the nerve fibers. It is our purpose in this section to trace the steps in the formation of the regenerated axons, restating briefly those observations already mentioned which are of significance in the final process and linking them together in a coherent whole.

1. Proliferation of axons in the central stump

On the first day after the lesion some of the axons grow out into the exudate and break up into many branches (fig. 16). Others on the first day give off fine branches from their surface within the sheath in the immediate neighborhood of the lesion (fig. 17), some of which find their way into the exudate. Thus, from the end of the first day on, fine nerve fibers, which are demonstrably branches of the medullated axons of the proximal stump, are present in the developing scar. The chief contribution to the new axons, which enter the scar from the cut end of the proximal stump, comes from the branching of the old axons at a somewhat later date (fig. 20). These side branches from the medullated axons are given off chiefly in the last 5 mm. of the proximal stretch; and, while a few are formed as early as the eighth day, they are constantly increasing in number to the thirty-fourth day. Running for the most part within the sheath of the old axon from which they arose, they arrange themselves into fascicles of parallel threads (fig. 24, *a*) when their course toward the periphery is direct, and into tangled skeins (fig. 24, *b*) when for any reason they fail to grow directly toward the periphery. Some idea of the enormous number of such branches can be obtained from a study of cross sections of the proximal stump just above the lesion (fig. 25). Thus within the space formerly occupied by one fiber there may be fifty or even more. These bundles of fine fibers, derived as branches from the old medullated axons, run compactly until they leave the old sheath and plunge into the scar. Here they scatter out in every direction. Some of these fibers never reach the scar but turn backward in the proximal stump. Whereever it is possible to see these fibers ending within the thickness of a section they are tipped with a bulb.

In the case of the non-medullated axons, which after an abortive regeneration undergo a cellulipetal degeneration for something more than one centimeter up the proximal stump, true regeneration begins about the fourteenth day. There occurs a downgrowth of new axons from above the point where the degeneration ceased. These new axons, on the growing ends of which small bulbs can be seen, grow toward the cut surface in the degenerated bundles. These new axons continue to increase in number until by the thirty-fourth day the bundles of non-medullated fibers in the last part of the proximal stump are larger and more compact than in a normal nerve. Although, because of the smallness of these fibers and the compactness with which they are grouped, it is not possible to demonstrate the occurrence of lateral branches on these fibers, there can be no doubt but that they have increased greatly in number. This is evident in the longitudinal sections, but even more so in transverse sections taken a short distance above the lesion (fig. 26, *a*). Here one sees large bundles of non-medullated fibers so closely grouped as to resemble a sympathetic nerve. Compare this drawing with figure 25 taken at a lower level and showing the multiplication of the branches of the medullated fibers. It will be seen that the medullated fibers in the neighborhood of the non-medullated bundles in figure 26 have not yet begun to give off branches. Hence the large increase in the non-medullated fibers cannot be due to the side branches of the medullated fibers growing into the bundles of non-medullated fibers. These bundles can be traced to the end of the central stump, where they go over into the scar intermingling with the branches of the medullated fibers from which they can no longer be distinguished.

This enormous increase in the number of fibers in the terminal part of the proximal stump is one of the most striking observations that have been made on these preparations—and it seems to the writer one of the most significant for the interpretation of the nature of the regenerative process. It fits logically into the scheme of the outgrowth theory. As we shall see, a great number of fibers fail to find their way through the scar to the

more favorable pathway formed by the distal stump, and the large number of branches increases the probability that at least one branch will reach that pathway and develop into a functional fiber. This multiplication of branches is, then, a means for compensating the loss of fibers in the scar. That more than one branch of a fiber of the central stump may make such connections and develop into a functional fiber is shown by the physiological experiments of Osborne and Kilvington ('08), who showed that when the peripheral stumps of the tibial and peroneal nerves were united with the central stump of the tibial nerve, and regeneration had taken place, it was possible by stimulating the peripheral end of the peroneal to obtain contractions in the muscles supplied by the tibial. These contractions could still be obtained after the tibial nerve had been divided above the point of the previous union, thus showing that some motor fibers in the central stump of the tibial had sent at least one branch into each nerve.

It seems probable, however, that at best only a few of the many branches of a central axon make connections with the distal stump and that the rest atrophy and disappear.

2. Penetration of the scar and loss of fibers

It is in the tracing of these new-formed fibers through the scar and into the distal stump that the study of the serial sections, in their serial order, was found of most advantage, since in them it was possible to demonstrate very convincingly the origin and course of these fibers.

As has been stated, the first nerve fibers make their appearance in the exudate on the first day. These are the fine non-medullated branches of the medullated fibers (fig. 16). No nerve fibers at any time grow out into the exudate or scar from the distal stump. After the first day little progress in the outgrowth of fibers is made for several days. A few fibers can be seen in the immediate neighborhood of the central stump on the second, third and fourth days, but in number and length they are not much in advance of those seen on the first day. This temporary arrest of the outgrowth of fibers is probably

associated with the violent reaction going on in the neurone at that time—the so-called 'axonal reaction.'

Unfortunately the eight-day specimen was divided into proximal and distal stumps by a cut through the scar near the central end before the tissue was prepared by the pyridine-silver method; and, as a result, the stain of the central portion of the scar is not good. On the fourteenth day, however, the scar is beautifully stained and fibers can be seen in large numbers leaving the central stump, plunging into the scar, and running through it in every direction. The same is true of the nineteen-day specimen. Here many fibers are seen working their way centrally in the thickened perineurium of the central stump in which they form a plexus connected with the plexus in the scar. These represent a portion of the aberrant branches that never find their way into the distal stump. Where fibers are seen to come to an end within the thickness of a section, they are tipped with a small bulb. On the twenty-fifth day (Dog x, in which union of the stumps was prevented by excision of 1 cm. of the nerve) the mass of scar tissue covering the end of the proximal stump is penetrated in every direction by these fine nerve fibers; from this mass covering the end of the central stump, they can be followed in gradually decreasing numbers upward in the thickened perineurium for 4 mm. and distalward in the scar for a distance of 13 mm. As one goes distally in the scar the number of fibers gradually decreases. No fibers reach the distal stump, and no fibers grow into the scar from the distal stump.

Figure 27 is drawn from the scar in the immediate neighborhood of the central stump of the specimen just described. It will be noticed that the fibers are arranged in bundles. The explanation of the formation of these bundles is the stereotropism demonstrated by W. H. and M. R. Lewis ('12) in growing fibers. When the growing tip of one fiber comes in contact with another fiber, it follows this second fiber just as, in the experiments of the authors just mentioned, growing fibers run along the under surface of the cover glass. It will be further noted that the bundles, while crossing each other in a more or

less irregular manner, run, for the most part, in a general direction from center (*c*) to periphery (*p*). This predominant direction is due to the chemiotactic influence exerted upon the growing fibers by the distal stump. There are in the field three branching fibers (*a*). Notice that in each case the centrally directed limb of the *Y* is the thickest fiber, while the two peripherally directed limbs are thinner, and represent the branches. Had these fibers arisen in situ, the branching fibers would in themselves be difficult to account for; but the fact that the central limb of the *Y* is almost always the largest of the three would be even harder to explain. There are no end bulbs represented in the drawing, and in fact there are few in this region. This is the oldest portion of the nerve plexus in the scar and the fibers are already of great length and few can be seen having a true termination within the thickness of a section. As one approaches the distal extremity of this plexus, 9 to 13 mm. from the central stump, these end bulbs become relatively much more numerous, since we are dealing here with the growing ends of the fibers. Often in the place of an end bulb one sees the end of a fiber breaking up into a large number of fine branches. It is an interesting fact that the farther distally in the scar the plexus goes the more parallel its fibers become, and the more directly they run toward the distal stump. This is explained by the fact that the earliest fibers penetrate the scar at a time when the distal stump has not undergone the alteration necessary for the exercise of the chemiotactic influence and their direction is very irregular. These early fibers by stereotropism govern the direction of the bundles. On the other hand, the early fibers in the distal portion of the plexus are from the first under the chemiotactic influence of the distal stump which is stronger because of the proximity of the distal stump.

Dog VIII, killed thirty-four days after the operation, serves as a good illustration of a scar when the two ends of the nerve have been sutured. The suturing was not well done and a gap of some size between the two stumps was filled with scar tissue. Into this scar the fibers from the central stump can be followed. The fiber plexus in the scar is very much like that in Dog X.

But in this case the plexus, composed in its distal part of fairly parallel fibers, is directly connected with the distal stump into which the bundles run in great numbers.

While of course it is not possible to follow individual fibers out of the central stump through the scar and into the distal stump, yet by a study of serial sections of different stages, it is possible to convince oneself that all the nerve fibers in the scar are outgrowths of central axons.

3. The utilization of the protoplasmic bands as pathways for the new axons in the distal stump

We have already described in the distal stump the multiplication of the nuclei of the neurilemma, the increase of the protoplasm which surrounds them, and the formation from these of nucleated protoplasmic bands. These bands are formed as the final stages in the degeneration of both medullated and non-medullated fibers. In none of the specimens was it possible to see any indications of the development of axons in situ. Occasionally one sees in them a pseudo-striation due to longitudinal rows of granules, but there are no transition stages between these and the sharply stained, regenerated axons. Negative results of this sort, obtained from preparations which sharply differentiate the finest branch of an axon, are in themselves of great significance. But even more important is the evidence of the direct growth into these protoplasmic bands of the fine nerve fibers of the scar.

In Dog v, killed fourteen days after the operation, and Dog vi, killed nineteen days after the operation, in both of which the cut ends of the nerve were united by sutures, nerve fibers had entered the distal stump from the scar. In the nineteen-day specimen they can be seen to run into the protoplasmic bands; but in the fourteen-day specimen these bands are not sufficiently well developed to permit one to say whether the new axons have entered these developing bands or not. In Dog x, killed twenty-five days after excision of 1 cm. of the nerve, the band fibers are well developed and clearly differentiated, but there is no trace of a new axon in the scar covering

the end of the distal stump, nor in the protoplasmic bands. Their absence in this case, twenty-five days after the operation, although well developed protoplasmic bands are present, and their presence in other specimens, fourteen and nineteen days after the operation when the protoplasmic bands are only incompletely formed, speaks strongly against their development in situ in these bands. Moreover, in both the fourteen- and nineteen-day specimens, nerve fibers from the central stump had reached the distal stump through the scar.

Probably the most instructive preparations, however, are those from Dog VII, killed thirty-four days after the removal of 1 cm. of the sciatic nerve. A study of serial sections shows that the scar covering the distal stump is devoid of nerve fibers except in one very limited area—here there are a few fibers. All of the protoplasmic bands in this specimen are devoid of new axons except a few in that part of the distal stump overlaid by the innervated portion of the scar. Here a few of the bands can be seen containing sharply staining axons. Figure 28 shows five bands in this location, down one of which a new axon is growing. The axon can be seen to end in an enlargement directed distally. The presence of a few of these sharply staining axons in protoplasmic bands adjoining the only part of the scar which contains nerve fibers, while all the rest of the bands are empty, is a strong point in favor of their growth into the distal stump from central fibers. The presence of end bulbs like the one seen on the fiber in figure 28 is evidence in the same direction.

Cajal lays stress on the occurrence of branching in the axons in the protoplasmic bands. Such branching has been observed occasionally in these preparations, but its value as an evidence of the down-growth of the axons seems to the present writer not to be very great.

In Dog VIII, killed thirty-four days after section of the sciatic with primary suture, great numbers of fibers from the scar run into the distal stump. Several axons often run down one protoplasmic band. Figure 29 represents a cross section of this distal stump. At *a* is seen a large protoplasmic band (formed

from a medullated fiber) containing five new axons, and at *b* a bundle of fine protoplasmic bands (formed from non-medullated fibers), in several of which new axons have appeared. All new axons lie in protoplasmic bands, never between them. It has been supposed by others that the axons may run between the bands as well as within them. This appears to be the case in longitudinal sections; but in cross sections one always sees the light yellow protoplasm of a band surrounding every axon. About some of these axons a faint halo of myelin is deposited by the thirty-fourth day.

No cases were studied in which sufficient time had elapsed for the complete regeneration of the nerve. We expect to make other experiments on young dogs with a view to determining the structure of a fully regenerated nerve and especially the relative proportion of medullated and non-medullated axons which it contains.

SUMMARY

1. The idea, first clearly stated by Büngner, that the new axons arise in the protoplasmic bands through a fusion of longitudinal striations which have developed in situ, has been shown by the application of the Cajal stain to be without foundation. The axons, when they first make their appearance in the distal stump, are fully developed and clearly differentiated from the surrounding protoplasm. They do not appear as discontinuous fragments, but as long fibers, which, when traced peripherally, may either run out of the section or end within a protoplasmic band with a terminal bulb, and when traced centrally either run out of the section or into a plexus of axons in the scar. In these findings all recent investigations agree. Perroncito ('05), Marinesco ('06), Poscharissky ('07), and Cajal ('08), who have used the Cajal stain, Pupura ('01) with Golgi stain, and Krassin ('06) with methylene blue, have all reached the same conclusion. Their results are in full accord with those of the present investigation. Even Bethe ('07) is silent on the question of the histogenesis of the regenerated axons in the distal stump, since in his last article he makes no attempt to defend his former views on this subject.

2. The attempts to obtain regeneration in a peripheral stump permanently separated from the spinal cord and spinal ganglia have led to negative results. So great is the regenerative energy of the central stump in young animals that the new axons to which it gives rise may bridge very great gaps to reach the distal stump; and new axons may grow into that stump from other nerves. Bethe's elaborate precautions to prevent such union have clearly not been adequate, as is shown by the experiments of Langley and Anderson ('04) and Lugaro ('05). Nothing short of the complete removal of the entire lumbo-sacral spinal cord with the associated spinal ganglia can be regarded as effectually preventing such reunion and these experiments have been negative. Since, however, such experiments are open to the objection that the high grade of marasmus caused by such an operation could in itself be sufficient to account for negative results, no very valuable evidence is likely to be furnished by this line of experimentation. Since we now have, in the Cajal method, a technique which enables us to see clearly how regeneration occurs when the two ends of the nerve are allowed to unite, there ceases to be any reason for attempting to secure an isolated stump. It is highly improbable that there would occur in such an isolated stump a process essentially different from that which occurs when the two ends are allowed to unite.

3. It can be shown by the Cajal stain that the axons of both medullated and non-medullated fibers in the central stump give rise to a large number of branches which make their way through the scar and enter the protoplasmic bands, which they utilize as pathways toward the periphery. Perroncito, Marinesco and Cajal have reached these same conclusions on facts similar to those presented in the preceding sections of this paper. Poscharissky, whose observations agree with those of the others, hesitates to draw any conclusions. The facts upon which the conclusions just mentioned are based have already been summarized under the heading "The mechanism of the regeneration of nerves."

4. The axons of the peripheral stump do not die at once after the division of the nerve, but live for two or three days at least, and undergo changes in the neighborhood of the lesion which must be regarded as an abortive regeneration. After a few days all the newly formed structures degenerate and disappear. This abortive regeneration has no significance for the ultimate regeneration of the nerve and is of interest only because of the light it throws on the life processes within the neurone. These phenomena, first seen by Perroncito, have been fully confirmed, not only in the present investigation, but also by Poscharissky, Marinesco and Cajal.

5. Alterations in the axons of the proximal stump can be noticed within twenty-four hours after the lesion. They consist of the formation of fine branches, and the rearrangement of the neurofibrils of the old axons to form the most complicated networks. These changes are limited to the immediate neighborhood of the cut surface and are too varied to summarize in detail. These were also first seen by Perroncito and confirmed by the others who have used the Cajal technique.

6. The new observations presented in this paper concern almost exclusively the non-medullated fibers which are now known to outnumber the medullated fibers in the spinal nerves. These observations have been summarized in the section on "The mechanism of the regeneration of nerves."

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PLATE 1

EXPLANATION OF FIGURES

The drawings were made from pyridine-silver preparations of the degenerating and regenerating sciatic nerve of the adult dog.

1 Ocu. 3, Obj. 2 mm. Degenerating medullated axon in the distal stump on the second day.

2 Ocu. 3, Obj. 2 mm. Two medullated fibers from the distal stump on the fourth day. Degeneration has progressed much farther in *a*, than in *b*. The neurilemma can be seen bounding the unstained myelin sheath.

3 Ocu. 3, Obj. 2 mm. Medullated fiber from the distal stump on the eighth day; *a*, fragmented axon surrounded by an elliptical segment of myelin.

4 Ocu. 3, Obj. 2 mm. Early protoplasmic band formed from a medullated fiber. From the distal stump on the nineteenth day; *a*, nucleus; *b*, droplet of myelin containing fragments of axon.

5 Ocu. 3, Obj. 2 mm. A bundle of degenerating non-medullated fibers from the distal stump on the second day.

6 Ocu. 3, Obj. 2 mm. Undegenerated non-medullated fiber from the distal stump on the fourteenth day.

7 Ocu. 3, Obj. 2 mm. Three protoplasmic bands formed from non-medullated fibers. From the distal stump on the nineteenth day.

8 Ocu. 3, Obj. 2 mm. Non-medullated fiber from the neighborhood of the cut surface of the distal stump, showing new formed lateral branch with end-bulb. End of first day; *c*, toward the center; *p*, toward the periphery.

9 Ocu. 3, Obj. 2 mm. Non-medullated fiber from the neighborhood of the cut surface of the distal stump, showing branching and two end-bulbs. End of first day; *c*, toward the center; *p*, toward the periphery.

10 Ocu. 3, Obj. 7. Medullated fiber from the neighborhood of the cut surface of the distal stump on the third day; *a*, disintegrated portion of the axon; *b*, club-shaped extremity of the living part of the axon; *d*, isolated neurofibril; *c*, toward the center; *p*, toward the periphery.

11 Ocu. 3, Obj. 2 mm. Medullated fiber from the neighborhood of the cut surface of the distal stump on the third day, showing fibrillar reticulum, isolated fibrils, and one fibrillar side branch with bulb; *c*, *c'*, toward the center; *p*, toward the periphery.

12 Ocu. 3, Obj. 2 mm. Formation of side branches and end-bulbs on the non-medullated fibers near the cut surface of the proximal stump at the end of the first day.

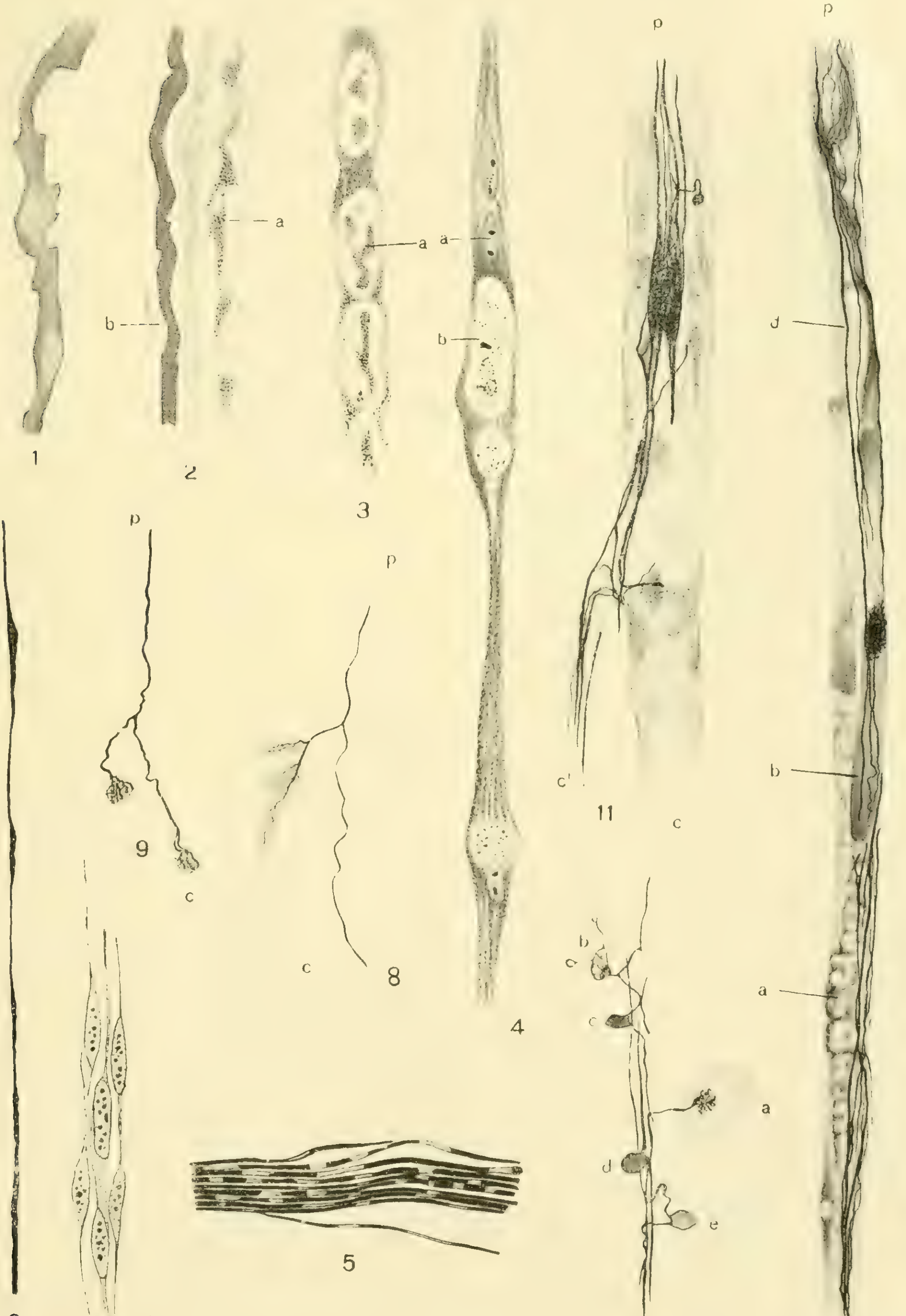
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PLATE 2

EXPLANATION OF FIGURES

13 Ocu. 3, Obj. 7. Bundle of regenerating non-medullated fibers in the proximal stump, on the fourteenth day; *c*, toward the center; *p*, toward the periphery.

14 Ocu. 3, Obj. 2 mm. Medullated fiber from the neighborhood of the cut surface of the proximal stump at the end of the first day; *a*, degenerated portion of the fiber; *b*, zone of reaction with neuro-fibrillar reticulum; *d*, only slightly altered portion of the axon; *c*, toward the center; *p*, toward the periphery.

15 Ocu. 3, Obj. 2 mm. Fibrillar dissociation in a medullated axon in the neighborhood of the cut surface of the proximal stump on the second day.

16 Ocu. 3, Obj. 2 mm. Two medullated axons, which have grown out from the cut surface of the proximal stump and given off fine branches in the exudate; *c*, *c'*, toward the center; *p*, *p'*, toward the periphery.

17 Ocu. 4, Obj. 2 mm. Medullated axon from the neighborhood of the cut surface of the proximal stump at the end of the first day. Many fine branches arise from its surface and end in small bulbs.

18 Ocu. 4, Obj. 2 mm. From the neighborhood of the cut surface of the proximal stump on the fourth day; *a*, obliquely cut medullated fiber with a tubular plexus of fine branches beneath the neurilemma; *b*, another tubular plexus derived from a medullated axon; *c*, row of degenerating non-medullated fibers and their end-bulbs.

19 Ocu. 3, Obj. 7. Plexus derived from a medullated axon. From near the cut surface of the proximal stump on the second day.

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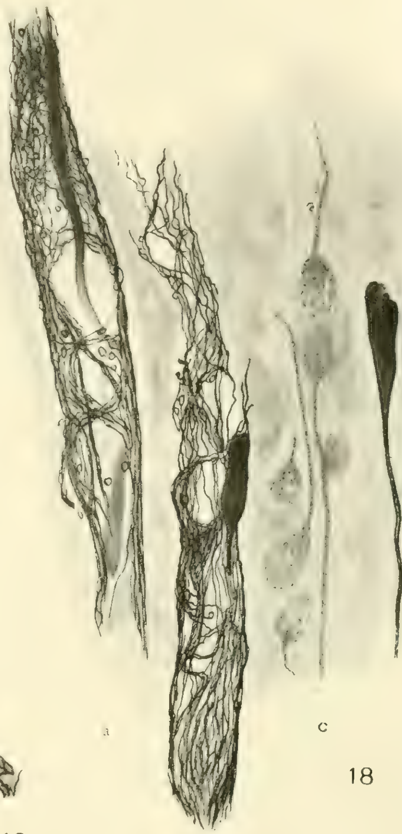

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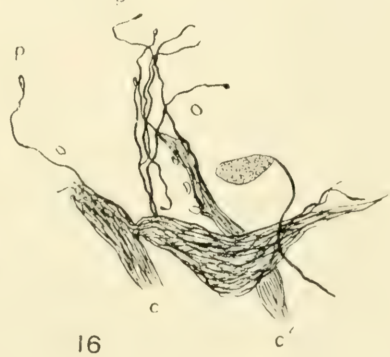

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PLATE 3

EXPLANATION OF FIGURES

20 Ocu. 3, Obj. 7. Branching of a medullated axon. From a point several millimeters above the cut surface of the proximal stump on the nineteenth day; *a*, point in old axon from which arises an extremely short branch which at once divides into two; *c*, toward the center; *p*, toward the periphery.

21 Ocu. 3, Obj. 7. Branches of a medullated axon of the proximal stump several millimeters above the cut surface on the twenty-fifth day; *c*, toward the center; *p*, toward the periphery.

22 Ocu. 3, Obj. 7. A branch on the side of a non-medullated fiber which might itself however be a branch of a medullated axon. From the proximal stump on the fourteenth day.

23 Ocu. 3, Obj. 7. Bundles of new axons in the proximal stump on the thirty-fourth day.

24 Ocu. 3, Obj. 7. A bundle and a tangled skein of new axons from the central stump on the thirty-fourth day; *c*, toward the center; *p*, toward the periphery.

25 Ocu. 3. Obj. 7. A cross section of three bundles and one tangled skein of new axons. From the central stump thirty-four days after the operation.

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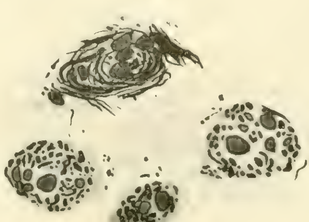

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PLATE 4

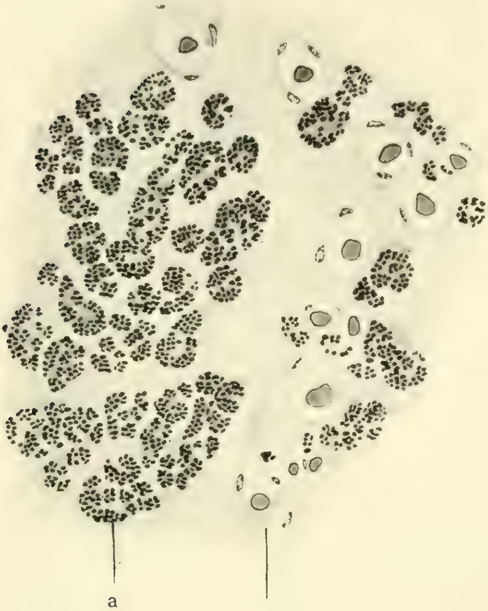
EXPLANATION OF FIGURES

26 Ocu. 3, Obj. 2 mm. From a cross section of the proximal stump on the thirty-fourth day showing the increase in the number of non-medullated fibers; *a*, bundle of non-medullated fibers; *b*, medullated fiber.

27 Ocu. 3, Obj. 2 mm. Bundles of new axons in the scar on the twenty-fifth day; *a* branching fibers; *c*, toward the center; *p*, toward the periphery.

28 Ocu. 3, Obj. 7. Five protoplasmic bands, down one of which a new axon is growing. From the distal stump thirty-four days after the operation; *c*, toward the center; *p*, toward the periphery.

29 Ocu. 3, Obj. 2. mm. Cross section of the distal stump on the thirty-fourth day; *a*, protoplasmic band from a medullated fiber containing five new axons; *b*, bundles of protoplasmic bands from non-medullated fibers some of which contain new axons; *c*, droplet of meylin in a protoplasmic band.



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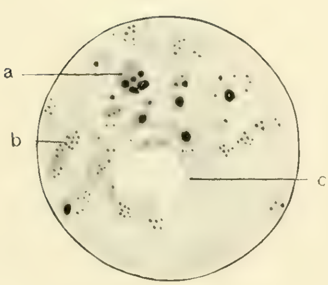

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THE CESSATION OF MITOSIS IN THE CENTRAL NERVOUS SYSTEM OF THE ALBINO RAT

EZRA ALLEN

From the Philadelphia School of Pedagogy and The Wistar Institute of Anatomy and Biology

TWENTY-TWO FIGURES

INTRODUCTION

While different observers have recorded the fact that mitosis continues in the central nervous system after birth in mammals which are relatively immature when born, the exact period of its cessation in any one such animal does not seem to have been determined. The purpose of this paper is to record results of studies which I have been pursuing, with many interruptions, for three years, in the effort to discover how long cell division may continue after birth in the central nervous system of the albino rat.

The literature on this phase of vertebrate growth is not extensive. Buchholtz ('90) records mitoses in all parts of the central nervous system of new-born dogs and rabbits and those a few days old. Sclavunos ('99) states that in new-born dogs, cats and white mice dividing cells are to be found in the wall of the central canal of the cord, in its white substance, in the substantia gelatinosa, in the anterior gray horns, at the point of entrance of the dorsal and ventral nerve roots, "among the cells of the spinal ganglia," in the dorsal and ventral septa of the cord, in the dura, and "abundantly" in the arachnoid and under the pia. Hamilton ('01) found mitoses in the cerebrum and spinal cord of the albino rat in animals four days old. Addison ('11), in his study of the Purkinje cells of the same animal, notes mitoses regularly occurring in the cerebellar cortex at twenty-one days, and in one individual at twenty-two days after birth.

MATERIAL AND TECHNIQUE

I am indebted to The Wistar Institute of Anatomy and Biology for the albino rats used in the study and for the use of its laboratory equipment. I am indebted to Dr. Stotsenbürg, of the Institute, for a supply of rats as needed and for courteous attention; to Dr. H. D. King for a method of imbedding and staining; to Dr. S. Hatai for valuable suggestions. To Dr. H. H. Donaldson I am especially indebted for constant aid and criticism.

My method of work was in general as follows. Healthy rats of the following ages were used: 1, 4, 6, 7, 12, 15, 18, 20, 30, 52, 70, about 120 days old, and one about two years old. In all, some twenty-five different animals were studied. Record was made of the weight, body-length and sex. After chloroforming the animal, the brain and cord were quickly removed, fixed in Carnoy's fluid and imbedded, the younger specimens in paraffin the older in celloidin and paraffin. Frontal sections were then cut at 8 micra and stained with either iron-alum-haematoxylin or thionin, followed by eosin or erythrosin. Thionin was found to differentiate the mitotic figures with sufficient clearness for this study. By using the mechanical stage, the sections were thoroughly explored with a magnification sufficient to detect mitoses. Only those cells which showed mitotic figures clearly were enumerated; the earlier stages of prophase, being more difficult to identify, were not considered. The Zeiss 4 mm. objective and No. 4 eyepiece usually sufficed; in doubtful cases the 2 mm. oil immersion objective and eye-pieces higher than No. 4 were employed.

Two methods of record were used. With the younger material, diagrams representing the sections studied were outlined and on these the approximate location of the dividing cells was indicated, the number *1* being employed for the cells in section No. 1, the number *2* for those in section No. 2, etc. (figs. 1 to 4). When the examination of a series was complete, this method showed graphically the distribution of the dividing cells in that portion of tissue both in cross section and longitudinally. The other method, usually employed for the older material, was to tally the cells as discovered opposite the number of the section, regional location being indicated by columns if desired.

All the figures are from frontal sections of the cord and brain of the albino rat.

Figs. 1 to 4 Diagrammatic drawings of frontal sections through the cervical cord and at three different levels of the cerebrum of a one-day-old rat. Each figure shows the position of a dividing cell in the section of the series corresponding to that number. The dotted lines outline gray matter. The ventral portion of the tissue appears at the bottom.

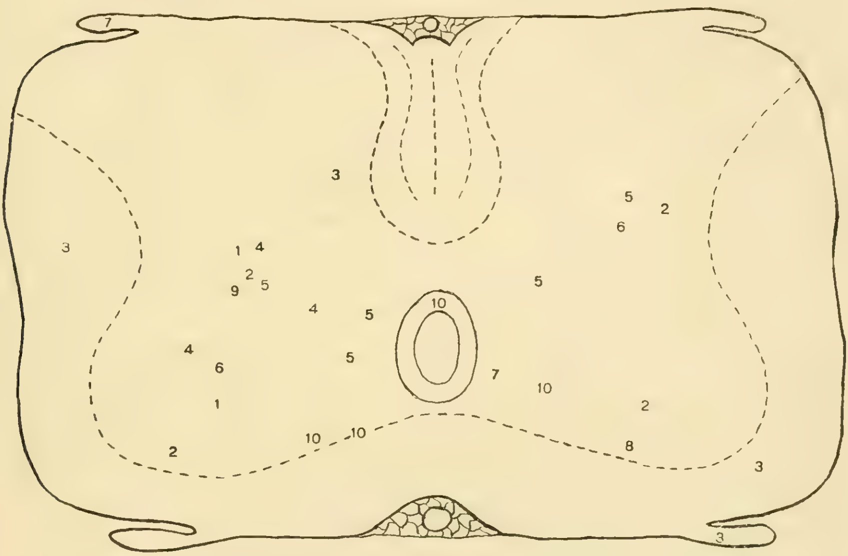
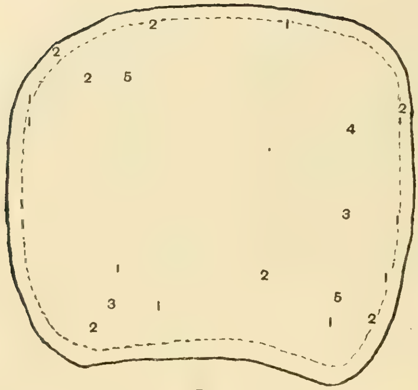


Fig. 1 Distribution of the dividing cells in ten consecutive sections of the cervical cord at the level of its greatest area. × 75.

As to the portions of the central nervous system studied. At first examination was made at various levels throughout the length of the cord, cerebellum and cerebrum to determine whether mitosis was limited to a particular locality. Then, in order to determine numerical relationships, attention was focussed upon certain definite levels. For the spinal cord, the sections showing greatest area in the cervical, thoracic and lumbar levels; for the cerebellum, sections passing through the region showing greatest area, and for the cerebrum sections passing through the optic chiasma were chosen. In each instance frontal sections were used. The number of sections thus studied varied a little—in the cord at least ten and frequently more: in the brain at least five and frequently more.



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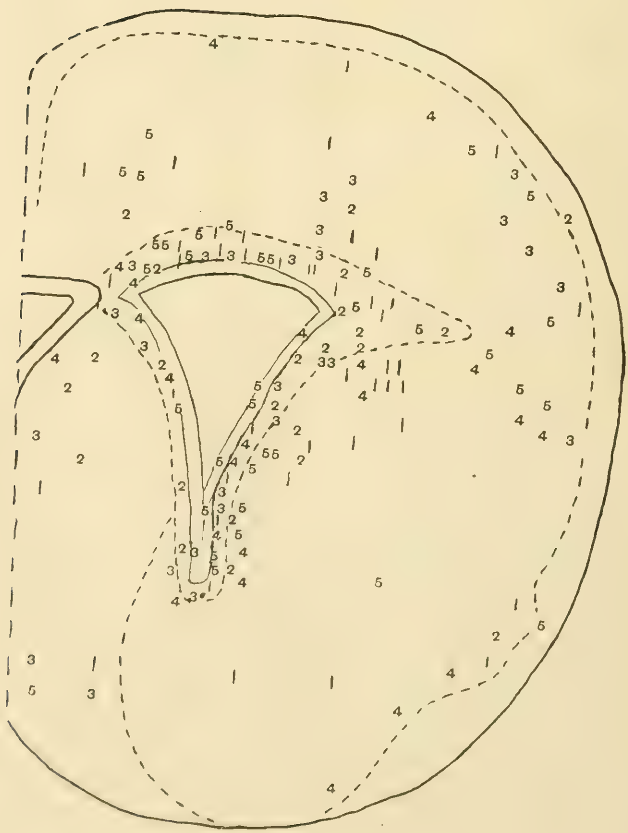

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Fig. 2 Distribution of the dividing cells in five consecutive sections cut through the frontal lobe of the cerebrum dorsal to the olfactory lobe. × 25.

Fig. 3 Distribution of the dividing cells in five consecutive sections cut through the optic chiasma. × 25.

Fig. 4 Distribution of dividing cells in five consecutive sections cut through the mid-brain and the occipital lobes of the cerebrum posterior to the lateral ventricles. × 25.

As a basis for determining the comparative rate of cell division the number of mitoses per cubic millimeter was obtained. With the aid of the camera lucida or projection apparatus, drawings were made of the first and last sections of a series. These drawings were then measured by the planimeter and the mean area taken. Since the sections were of a fixed thickness (8 micra) the volume of the tissue in question was readily calculated. The number of dividing cells in the series of sections had already been determined, so that the necessary data for finding the number of dividing cells per cubic millimeter of tissue were at hand.

STATEMENT OF RESULTS

The distribution of mitoses

A brief statement will first be made under this head to be followed by more detailed explanation. The results of this study show that at birth mitoses are occurring in each division of the central nervous system, at all levels of the cord, cerebellum and cerebrum. An examination of figures 1 to 4 will make it evident that the general distribution, however, is unequal. Table 2 (p. 557) indicates at different ages the relative mitotic activity per cubic millimeter at the three principal levels of the cord and at one level of the cerebellum and cerebrum respectively. Figures 2 to 4 show the relative longitudinal distribution at three levels in the cerebrum, as well as the fact that in this organ the region of greatest activity is in the walls of the lateral ventricles. In the cerebellum dividing cells are most abundant in the cortex. Table 2 shows the relative rate of mitosis at different levels for the ages from one day to twenty-five days after birth. This table shows also that cell division stops earliest in the cord, a little later in the cerebellum and still later in the cerebrum. With these data in mind we may pass to the details.

1. The distribution as it appears in frontal section will be better appreciated by a preliminary consideration of the embryonic differentiations of tissue as they appear in the central nervous system. The original epiblastic layer forming the wall of the neural tube is early converted into a protoplasmic framework

(myelospongium) which has a columnar and radial disposition and shows three layers: (a) the innermost or germinal zone; (b) a middle nuclear or mantle zone crowded with daughter cells which have migrated from the actively mitotic germinal layer; and (c) an outer nuclear-free reticular or boundary zone. (His '89 and '04; Hardesty '04; Bryce '08). The seat of mitotic activity is confined at first to the germinal zone but later spreads to the mantle layer (Hamilton '01; Hardesty '04). Activity continues in these two layers with increasing age, but becomes more rapid in the latter (the middle nuclear or mantle). Hamilton ('01), writing of the spinal cord of the albino rat, states: "As

TABLE 1

Giving the percentage of ependymal mitoses in the spinal cord of the albino rat from one day to fifteen days old, based upon the number of mitoses in ten sections from the cervical, thoracic and lumbar levels respectively, excepting that in the fifteen-day rat twenty sections at each level were used.

AGE	TOTAL NUMBER OF MITOSES	EPENDYMAL MITOSES	PERCENTAGE OF EPENDYMAL MITOSES
days			
1	47	4	8.5
4	96	0	0.0
6	115	6	5.2
12	22	2	9.0
15	4	1	25.0
Totals......	284	13	4.5

The percentage of 13 to 284 is 4.5, the percentage of the ependymal mitoses to the total number of mitoses during the period from 1 day to 15 days.

development proceeds, there is a relative increase of extra-ventricular mitoses, so that by the end of the first day after birth they are greatly in the majority." My preparations from the one-day cord of the same animal confirm this observation. At this age the mitoses are distributed in the ependyma, in the white and gray regions, in the fiber tracts, in the nerve roots, in the enveloping membranes and among the spinal ganglion cells. With advancing age the distribution appears as shown in table 1, the data for which were obtained by adding together the number of mitoses found in ten sections taken from the cervical, thoracic

and lumbar regions of the cord respectively, with one exception, viz., that twenty sections instead of ten were used from each region as a basis for the figures for the fifteen-day specimen.

Hamilton ('01), from observations on twenty-five consecutive sections from the lumbar cord of one new-born rat, concludes that the greatest number of mitoses is in the anterior gray column. This relationship apparently does not persist during the later stages of mitotic activity, as shown by my sections. Furthermore the animals which I have studied show great individual variation at the same age. In addition, the same animal varies greatly in its number of mitoses in series of sections taken at only short distances apart in the same approximate level.

The ependyma of the cerebellum shows very few mitoses in the one-day-old animal. In the cortex they are abundant; there are few in the intermediate tissue. This condition prevails throughout the length of the organ. By the age of twelve days cell division is confined to the cortical area and does not reappear in other portions.

The cerebrum shows a distribution the reverse of that found in the other two divisions. While at birth it is diffuse, the greatest amount of activity is seen in the germinal and mantle layers about the lateral ventricles (fig. 3). The activity external to these layers grows less until at the age of twelve days there is scarcely any cell division elsewhere. Furthermore, the activity about the lateral ventricles is not equally distributed. It is least on the ental lateral surface and the ventral portion of the ectal wall; greatest along the roof and dorsal portion of the ectal lateral wall, where the mantle layer is widest (fig. 7). This distribution is to be seen in the animal one day old at whatever level of the ventricle we may section. In the older animals the region of activity becomes limited to the small portion of the ectal lateral wall lying a little ventrad from the point of union of this wall and the roof. (See further discussion of this zone under the topic Structural Correlations).

2. A comparison of sections from the same rat taken at different levels shows that the rate of mitosis is not equal throughout the length of the central nervous system. The cord is the region

of the least and the cerebellum the seat of the greatest activity, while the cerebrum in that portion where cell division is most rapid exhibits an activity somewhat greater than that of the cord. Table 2 (p. 557) contains figures illustrating these different rates. In addition, mitotic activity is unequal at different levels of the cord and cerebrum. Table 2 brings out this relativity for the cord. The rate of cell division is seen to be lowest in the thoracic and highest on the whole in the cervical portion. In the latter it rises on the sixth day to a rate equal to the most rapid exhibited by the cerebrum (on the fourth day), and more than twice as rapid as that of the cerebrum at six days. From a comparison of numbers of dividing cells at the three levels of the cerebrum, it is found that the greatest number is in the region of sections which pass through the optic chiasma.

This unequal longitudinal distribution is still further illustrated in a small way within the limits of each level, as shown by the markedly greater number of mitoses to be found in two or more consecutive sections of a series, this greater number standing out prominently from the relatively smaller number in the sections immediately preceding and following. Three illustrations follow, chosen from many which show the same relationship. The figures representing the number of mitotic cells in each section of the series run as follows: (1) from the cerebrum—4, 5, 7, 9, *11*, *16*, *14*, *21*, 3, 9, 3, 9, 3, 4; and (2) from the cervical cord 4, 0, 2, 3, *5*, *8*, 1, 4, 2. The numbers italicized in each series mark this grouping of mitotic activity, which may be termed a 'locus' of activity. Similar loci appear in the cerebellum. (3) A brilliant illustration presented itself in the cerebrum of a twenty-five-day specimen, an age when the mantle layer is not so crowded with nuclei as it is in younger material. This illustration will also show the tendency of cells to form groups and to retain a group identity. In this instance three well-marked groups of closely-packed, densely-staining nuclei were found, each well differentiated from the surrounding nuclei, and each showing mitoses. (See mn^1–mn^3 in fig. 10; enlarged view of one group is to be seen in fig. 21). These groups extended through three consecutive sections of 8 micra. I have not endeavored to ascertain

whether such loci are constant in their appearance at fixed points of the system or vary with different individuals. The large number of such loci found within small portions of the tissue at all ages and in each individual examined shows the necessity of using a considerable number of consecutive sections if one desires to make wide application of figures obtained from any one level.

3. Mitosis continues for different periods after birth in the different levels. It ceases first in the cord. Very few dividing cells are to be found in the fifteen-day cord at any level, as shown in table 1 (p. 553). No dividing cells were found in two eighteen-day-old specimens, although sections widely distributed in the different levels respectively were examined. No mitoses were found in the cords of older animals. The eighteen-day period may then be regarded as the time when mitosis has ceased in this portion of the system, having ceased between this and the fifteen-day period.

In the cerebellum it continues until some time between the twenty-second and twenty-fifth day, while in the cerebrum it continues still longer—to a slight degree in material about 120 days old, further discussion of which is taken up under the topics which follow.

4. Table 2 shows the distribution of dividing cells per cubic millimeter at three levels in the spinal cord and at one in the cerebellum and cerebrum respectively. The data for these figures come from consecutive frontal sections at the different levels as fully described in the introduction (p. 552). Each number in the table is not an average from several different rats but is taken from the records of one individual. For a given age, the numbers italicized are from the same animal. It will be noticed that the figures for the cerebellum in each of the animals of the first three ages are not from the same animals as those which furnished the figures for the other levels. This substitution is due to certain technical difficulties which presented themselves, such as failure to get good sections or to obtain true frontal sections in the desired locality. These three cerebella are the only ones of these ages upon which estimations were figured; others might have furnished slightly different figures.

TABLE 2

Showing the number of mitoses per cubic millimeter of nerve tissue in the central nervous system at certain levels. The figures are taken from calculations of the volume of tissue and the number of mitoses in ten consecutive sections at each level of the cord, five in the largest portion of the cerebellum and five in the cerebrum in the region of the optic chiasma (see p. 552). The numbers italicized are from the same individual of that age. The letters (a), (b) and (c) refer to different rats of the same age.

AGE	CORD			CEREBELLUM	CEREBRUM
	Cervical	Thoracic	Lumbar		
days					
1	*208*	*115*	*259*	1597	*430*
4	*437*	*176*	*351*	2111	*447*
6	*446*	*236*	*320*	(7-day)4848	*193*
12	*46*	*75*	*14*	*839*	*37*
20	00	00	00	(*a*) *00*	(*a*) *18*
20	*00*	*00*	*00*	(*b*) *61*	(*b*) *27*
20	00	00	00	(*c*) 520	
25	*00*	*00*	*00*	*00*	*27*

The differences of quantitative distribution which appear from this table if one reads the figures from left to right (according to the same age) have been noted on page 555. If one reads the columns downwards, it appears that the rate of cell division increases after birth (a confirmation of Hamilton '01) until the sixth day in the cord and the seventh in the cerebellum, but in the cerebrum only until the fourth day; after these respective dates the rate decreases rapidly. Cell division has ceased in the cord at the twentieth day, in the cerebellum at the twenty-fifth day, but in the cerebrum is still continuing at this last age at the same rate as for the twentieth day in specimen *b*.

Since these figures are taken from so limited a number of animals of each age, and from a length of only 80 micra in the cord and 40 micra in the brain at each level, they are to be interpreted as representing the general movement of mitotic activity, not as furnishing the basis for an accurate curve which will show the rate of mitosis after birth. More data must be gathered before such a curve can be constructed. However, the wide difference at each age between the rate in the cerebellum and the other levels indicates that we are safe in concluding that this organ presents the greatest degree of activity. There is good

reason for this condition since at birth the cerebellum is developed to only a slight degree, and its growth thereafter is very rapid during its first twenty-five days of life.

Hamilton ('01) found that the rate of cell division is less at birth than before birth. Unfortunately her paper does not give the embryonic age at which the rate is highest. Otherwise we might fix approximately the two periods in the life history of the albino rat when this factor of growth is most important in the cord and cerebrum. Since the figures given by her are not in terms of number per cubic millimeter, we have not the data needed for an exact comparison of the two phases of growth.

STRUCTURAL CORRELATIONS

1. Correlations in the spinal cord

If we compare sections of the cervical cord from one-day and twenty-day rats respectively, we see several morphological differences (figs. 5 and 6). (1) In the first place, the wall about the central canal of the one-day animal shows germinal cells in mitosis, and shows also that its myelospongium at each end lacks nuclei. Neither of these phenomena appears in the older material. In addition, the form of the canal in the older differs from that of the younger in that it approaches more nearly the strongly elongated oval assumed in the adult cord. (2) The number of cells in the immediate vicinity of the canal of the twenty-day animal is less than in the one-day old. (3) There is greater degree of maturity in all the cells of the older as indicated by their size and cytoplasmic development. And (4) the number of migrating cells is much less in the older than in the younger animal, although the region of greatest migrating activity remains the same in both ages—the region just ventral to the canal.

In most vertebrates the germinal and mantle layers about the canal have been completed some time before birth. One known exception is the chick. Merk ('86) figures the cord of a chick of seven days and five hours old which shows a nuclear-free space in the germinal zone at each end of the canal, a condition found in the albino rat at six days.

Figs. 5 to 11 Untouched photographs of frontal sections through the cord and cerebrum of albino rats of different ages. They show the ventral side downward on the page. The letters indicate the same structures throughout: *ventr*, ventral; *ect*, ectal; *ent*, ental; *r*, roof; *V.iii*, third ventricle.

Fig. 5 One-day rat, cervical cord. × 84.

Fig. 6 Twenty-day rat, cervical cord. × 84.

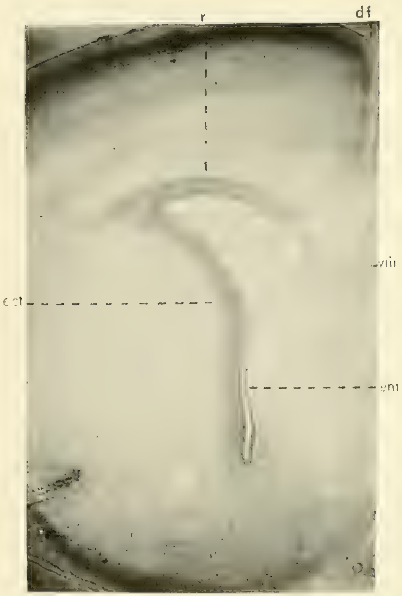
The germinal layer is completed progressively in the different levels of the cord in the albino rat. The completion is earliest in the thoracic region, where it is fully formed by the twenty-day period. It matures next in the lumbar region, where the wall is closed in nearly every section of the twenty-five-day and in every section of the thirty-day-old animals. In the cervical region, finally, a few sections at the last-named age still show a nuclear-free myelospongium at the ventral portion. The dorsal end is closed at all levels in the interval between the six-day and twelve-day periods.

2. Correlations in the cerebellum

The most interesting feature here is the external granule layer. Mitosis continues in this layer after it has ceased in all other parts of this organ. It ceases when the inward migration of the nuclei has taken place, a movement which is usually completed between the twentieth and twenty-fifth day (Addison '11). I found that in one specimen examined the migration had been completed at twenty days. Addison ('11) found in one of his preparations dividing cells in this layer at twenty-two days. Examination of two twenty-five-day specimens prepared by me revealed no mitoses; neither was any trace of the external granule layer present.

3. Correlations in the cerebrum

The well developed and persistent mantle zone lying along the ectal wall of the lateral ventricles is of particular interest, for in it occur the dividing cells found in the oldest specimens which show mitoses, and this layer, unlike the external granule layer of the cerebellum, never entirely disappears in animals up to two years of age, the oldest which I have examined (fig. 9). The one-day and twenty-five-day specimens both show dividing cells at every level along the entire length of the ventricles. In each case they are most abundant at the level of the optic chiasma. The mantle zone, while in general following the outline of the ventricular wall, is not of the same thickness throughout in any



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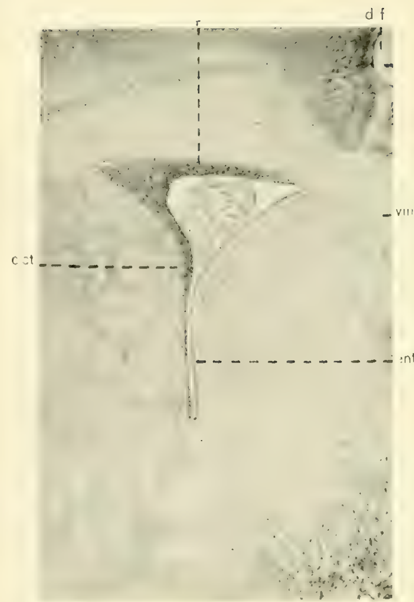

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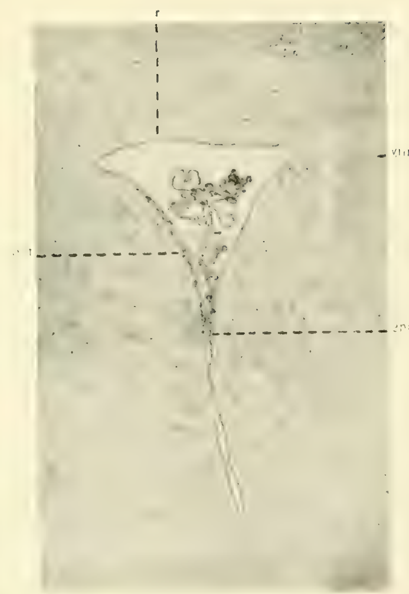

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Fig. 7 One-day rat, cerebrum; (one hemisphere) frontal section through optic chiasma to show the condition of the ventricular wall. × 21.

Fig. 8 Six-day cerebrum, as in figure 7. × 21.

Fig. 9 Two-year cerebrum, as in figures 7 and 8. × 21.

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Fig. 10 Twenty-five day cerebrum, dorsal portion of the lateral ventricle to show the mantle zone on a larger scale. $\times$ 84. The letters mn^1, mn^2 and mn^3 refer to loci of active cells (see p. 555).

Fig. 11 Two-year cerebrum, as in figure 10. $\times$ 84.

frontal section (figs. 7 to 11). It is widest at the point where the ectal lateral wall and the roof meet, and wider all along the ectal lateral wall than along the ental wall. In the one-day animal the layer extends all around the ventricle, but, beginning at the ventral portion, it is gradually reduced in extent until at twenty-five days old it consists of a single layer on the ental lateral wall; on the roof it is in almost the same condition, while on the dorsal portion of the ectal lateral wall and extending well outward at the point it is still several cells in thickness (fig. 10). Reduction is nearly complete along the ental lateral wall at the six-day period (fig. 8). In the twenty-five-day specimens cell division is occurring only in the portion of the ectal lateral wall and roof between mn^1 and mn^3 in figure 10. In this region a total of eight dividing cells was found in a seventy-day animal in five frontal sections at the level of the optic chiasma, these being along the ectal lateral wall, with none on the roof.

The mantle layer persists in this limited region along the ectal lateral wall in the two-year animal, as shown in figures 9 and 11. The active portion of this layer continues *in advanced age* to occupy the region a few cells in width external to the germinal layer, as shown by figures 21 and 22. There is evidently some connection between this layer and the "Uebergangsschichten" of His ('04) shown in his section of the human foetal cerebrum of four months.

NATURE OF THE DIVIDING CELLS

The tissues ultimately found in the nervous system are the neurones, the neuroglia and the connective tissue. In addition we find occasional leucocytes and a relatively greater number of lymphocytes. Hardesty ('04) from his study of the developing neuroglia of the pig, concludes that, "With the present technique there is nothing to show that all the products of the mitoses (germinal cells) in the ependymal layer are not indifferent elements from the first—capable of developing into either neurones or neuroglia." The neuroglia tissue has a double source of origin, arising from both the ectoblast and mesoblast. Hatai ('02) con-

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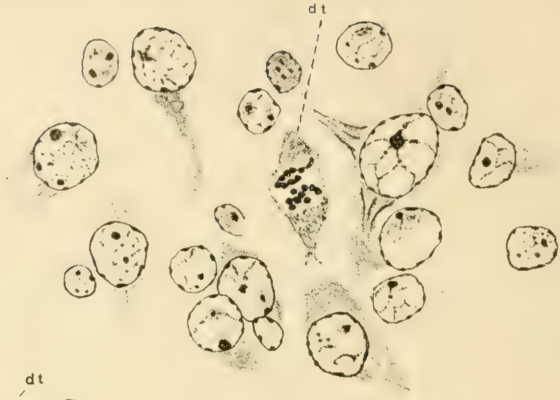

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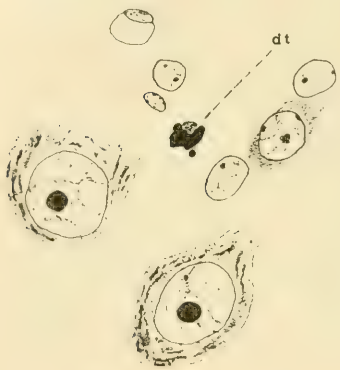

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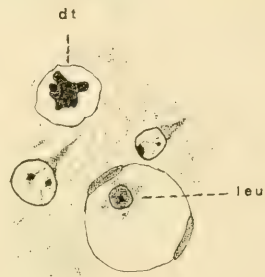

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Figs. 12 to 22 Camera lucida drawings of portions of tissue in cord and cerebrum chosen to show dividing cells. All the drawings were made with the Zeiss 2 mm. oil immersion lens and No. 6 eye-piece, tube length 176 mm. The drawings have been uniformly reduced to make the magnification as printed equal 800 diameters. The letters indicate the same structures throughout: *mli*, membrana limitans interna; *g*, dividing cell in germinal layer; *mn*, dividing cell in mantle (middle nuclear) layer; *ep*, ependymal cells; *dt*, cells in differentiated tissue; *leu*, leucocytes; *ca*, cell of capillary wall.

Fig. 12 Four-day rat, cervical cord; dividing cell in germinal layer. × 800.

Fig. 13 Six-day thoracic cord; large dividing cell in posterior horn. This cell is one of two dividing cells very near each other, each of which showed its chomosomes in three consecutive sections of 8 micra each. × 800.

Fig. 14 Twelve-day thoracic cord; small dividing cell in posterior horn. × 800.

Fig. 15 Twelve-day thoracic cord; mesodermal dividing cell of capillary found in postero-lateral tract; same section as figure 14. × 800.

Fig. 16 Six-day cerebrum, cortex; leucocyte within a cross section of capillary and a dividing cell. It is difficult to determine whether the granular mass about the nucleus of the leucocyte is its cytoplasm or other material. × 800.

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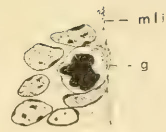

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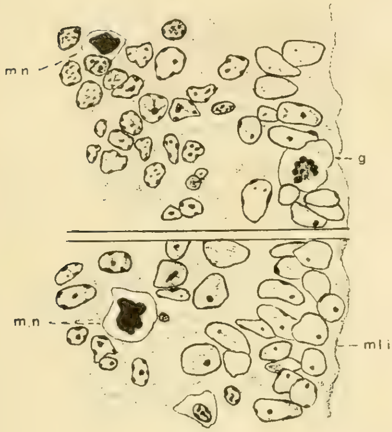

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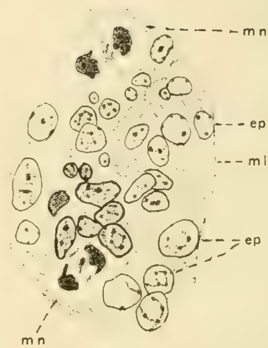

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Figures 17 to 22 are from the cerebrum of rats of different ages, showing portions of the zones bordering the ectal lateral wall of the lateral ventricle at about the locality marked mn^2 in figure 10, with the exception of figure 19, which is from the cortex.

Figs. 17 and 18 One-day cerebrum; small portions of germinal layer separated from each other by only a few cells. × 800.

Fig. 19 One-day cerebrum; small portion of the cortical region of the same section as figures 17 and 18. × 800.

Fig. 20 Six-day cerebrum; adjacent portions of germinal and mantle layers. × 800.

Fig. 21 Twenty-five-day cerebrum; two dividing cells in mantle layer, the lower at the edge of a group of cells (mn^2 in fig. 10). (See p. 555.) × 800.

Fig. 22 120-day cerebrum; germinal and mantle layers and two cells showing cytoplasm in the first layer of differentiated tissue. At this age the ependymal cells are clearly differentiated; they began to appear in the twenty-five-day material, as shown in figure 21. The reduction in the number of cells in the two inner layers is to be noticed. × 800.

firms the observations of Fragnito and Capobianco with regard to the mesoblastic source:

> From these observations the conclusion is drawn that the neuroglia nuclei in the white rat as well as in the mouse represent two distinctly characterized types: namely, nuclei the structure of which resembles very closely that of the nerve cells, and the nuclei the structure of which resembles very closely that of endothelial cells which form the capillary wall. These two types of nuclei have been derived from the ectoblast and mesoblast respectively. The latter type has probably two sources of origin; that is, they are partly derived from mesoblastic cells immigrating from the meninges (Capobianco and Fragnito), and partly from proliferating endothelial cells of the walls of the capillaries, these cells having separated from the capillary wall and migrated into the surrounding tissue, where they constitute one type of the neuroglia elements.

Hamilton ('01) differentiates two types of dividing cells in the cerebrum and spinal cord, the one, large with cytoplasm well developed and the chromosomes more scattered, the other small without cytoplasm and with the chromosomes solidly bunched. The former, she thinks, develop into nerve cells and the latter into neuroglia. Hardesty ('04) is of the opinion that the neuroglia cells cannot be differentiated from the inward-migrating white fibrous corpuscles. My observations lead me to agree with Hardesty, and so far as my methods of staining reveal any differentiations there seems to be no way of determining whether the small dividing cells are to become neurones, neuroglia cells or white fibrous corpuscles, since the small neurones which show processes very plainly are no larger than some of the dividing cells (figs. 14 and 16).

My preparations indicate that division in the two sizes of mitotic cells does not continue to the same period in the cord and cerebrum. In the cord, the large cells are found dividing in various regions up to the sixth day (fig. 13). While twelve-day specimens still show some mitoses in the germinal zone, the dividing cells of the extra-ependymal regions possess the characteristics of the smaller type. In the cerebrum the chromatic mass in the dividing cell measures up to 7.5 x 8 micra in the younger material, while in 70-day and 120-day material, a list of measurements runs as follows, the tissue from the two animals having

been prepared in the same manner: 70-day—5 x 4, 5.5 x 5, 6 x 4, 6 x 3 micra; 120 day—6 x 4, 4 x 5, 4 x 4 micra. None were found measuring more than 6 x 4 micra.

The dividing cells which I have enumerated in this study are believed not to be leucocytes or lymphocytes for the following reasons: the size of the nuclei in leucocytes found in blood vessels in my preparations is from 3.2 x 3.2 micra to 3.8 x 3.8 micra, while the chromatin material in the nerve nuclei measures from 4 x 4.2 in anaphase to 7.5 x 8 micra in prophase and metaphase. Moreover, after the dividing cells have disappeared from all other areas they are still to be found in the limited zone along the lateral ventricles of the cerebrum which has already been described. They are to be found also in the neighborhood of capillaries but this association is not constant.

The differentiation from lymphocytes is easier. These are found scattered through the tissue; they are smaller than the leucocytes and are readily recognized by the characteristic nuclei, their chromatin being gathered into one, two or more well separated, densely stained spherical masses, the outlines of which are always smooth and regular, lying in a clear nuclear matrix bounded by a limiting membrane of uniform thickness.

CONCLUSIONS

1. Mitosis ceases in the central nervous system of the albino rat as follows: (a) No mitoses are found in the cord at any level after the eighteenth day. This is somewhat previous to the complete cellular differentiation of the wall of the central canal, a stage accomplished in the thoracic region by the twentieth day after birth, in the lumbar region by the thirtieth day after birth, while at this last-named age the cervical level shows in occasional sections a portion of the wall still incomplete. (b) In the cerebellum mitosis ceases when the migration of the cells in the external granule layer is complete, a condition reached between the twentieth and twenty-fifth day after birth. (c) In the cerebrum mitosis continues with a considerable degree of activity to the twentieth

day after birth, after which it is found to a slight degree in the mantle layer along the ectal lateral wall of the lateral ventricles, this layer persisting at least to the age of two years. The latest observation of dividing cells in this locality was in 120-day material.

2. The rate of mitosis increases for a time after birth, reaching its high point at about the seventh day for the cord and cerebellum and about the fourth day for the cerebrum.

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SUBJECT AND AUTHOR INDEX

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